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These papers are contributions No. 192-200 from the Botanical Laboratories of Butler University.

Address all communications regarding them to Butler University Botanical Studies, Butler University, Indianapolis 7, Indiana, U. S. A.

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NOTES ON THE ALGAE—III

By William A. Daily

The specimen cited here is to be found in the Herbarium of Butler University and the Cryptogamic Herbarium of the Chicago Natural History Museum.

MYXOPHYCEAE—III

Porphyrosiphon Notarisii (Menegh.) Kütz. ex Gom., Ann. Sci. nat. VII Bot. 15:331. 1892; Drouet, Amer. Jour. Bot. 24:601. 1937.

This member of the Oscillatoriaceae apparently heretofore has not appeared in the literature on Indiana algae. It is rather widely distributed in North America, particularly in the eastern and middle western United States. Specimens are on file in the Chicago Natural History Museum from Pennsylvania, New Jersey, Maryland, Vir-

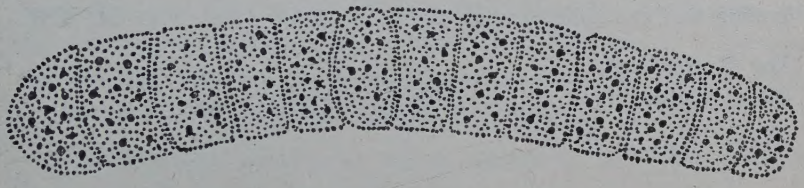


Fig. 1. Mature filament showing sheath structure.

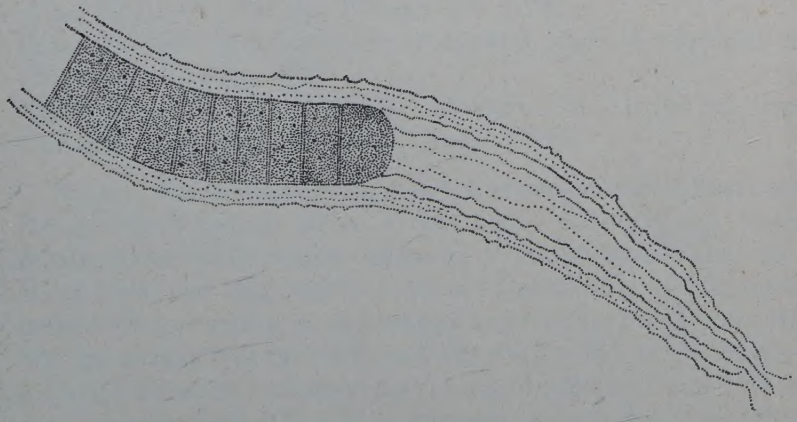


Fig. 2. Hormogonium.

ginia, West Virginia, North and South Carolina, Georgia, Florida, Wisconsin, Iowa, Illinois, Ohio, Arkansas, Nebraska, Kansas, Oklahoma, Missouri, Louisiana and Texas. Indiana is now added to this list.

For a taxonomical and morphological discussion of this Myxophycean see F. Drouet, "The Brazilian Myxophyceae I," Amer. Jour. Bot. 24:598-608. 1937.

The plant mass formed an expanded thin layer on the soil. The filaments are unbranched, variously curved, densely entangled; sheaths reddish-brown, often colorless at the apex, sometimes showing layers of different colors, the outer ones colorless, firm, becoming very thick, lamellose, with the apex attenuate and fibrillose, coloring blue when treated with chlor-zinc-iodine; trichomes 8-15 microns wide, usually constricted at the cross-walls, generally only one within the sheath, apical cell tapering, obtuse; protoplasts 4-10 microns in length, granulated and blue-green. Figures 1 and 2 drawn from herbarium specimens.

Specimen seen: Indiana: Montgomery county: On soil in road leading to Pine Hills, Fay K. & W. A. Daily *1442,* May 11, 1946.

The Herbarium
Butler University

NITELLA CAPILLATA A. BR. IN NORTH CAROLINA

By Fay Kenoyer Daily

Apparently until its collection by Dr. L. A. Whitford of the University of North Carolina at Raleigh, the species, *Nitella capillata* A. Br., was known only from the type. Furthermore, the type collection has not been found by the author. Probably, it was lodged in the herbarium of Dr. A. Braun in Berlin where it may have been destroyed during World War II.

Thanks are extended to Dr. Ray C. Friesner, Dr. Francis Drouet, Dr. Richard D. Wood, Mr. Sidney Esten, and Mr. W. A. Daily for their assistance in this study.

Specimens will be found at Butler University, the Cryptogamic Herbarium of the Chicago Natural History Museum, the Farlow Herbarium at Harvard, the Missouri Botanical Garden, the New York Botanical Garden, the United States National Herbarium, and the personal herbarium of the author. Dr. Whitford is to be commended for collecting a copious amount of material some of which arrived in a fresh condition for detailed study.

The following description agrees essentially with that given by Dr. A. Braun except where indicated:

Nitella capillata A. Br. Die Characeen Afrikas, Monatsb. Akad. Wiss. Berlin for 1867, pp. 797 and 893. 1868.

Braun, A. and Otto Nordstedt. Fragmente einer Monographie der Characeen. Berlin. 1882.

Nordstedt, Otto. Die Algis et Characeis 3-6, Aftryck af Lunds Universitets Ärsskrift XXV:14. Lund. 1889.

Habit: lax sterile verticels at which fertile gelatinous heads arise, not greatly branched, about 25 cm high. Monoecious. Stem: usually about 0.5-0.7 mm in diameter. Sterile branchlet: up to about 30-40 mm long; 6-8 in a verticel (mostly 6); 3-4 furcations; number of rays arising at succeeding furcations are 6 or 7 at the first, 5 at the second, 4 at the third, and 3 at the fourth; measurements of a typical branchlet—primary ray 0.4 mm in diameter and 14.5 mm long, secondary ray 0.27 mm in diameter and 7.9 mm long, tertiary

ray 0.16 mm in diameter and 2 mm long, quaternary ray 0.05 mm in diameter and 1.6 mm long, quinary ray 0.025 mm in diameter and 3.7 mm long; ultimate ray 3-4 celled; ultimate cell variable from pointed to blunt (in Braun's description these are blunt). FERTILE VERTICEL: of 6-8 branchlets (mostly 6); about 5 verticels produced one above the other forming a head enclosed in a gelatinous sheath interrupted at the base. FERTILE BRANCHLET: 1-2 mm long; 2-4 furcations (mostly 3); number of rays arising at succeeding furcations are 5-7 at first, 4 or 5 at second, 4 or 5 at third, 3 at fourth; 2 or 3 celled (mostly 3); ultimate cell variable from blunt to pointed (Braun's description—tip rounded, blunt). OOGONIUM: produced singly in all furcations; cells of coronula about 0.016 mm broad, upper series 0.021 mm long, lower series about 0.011 mm long. OOSPORE: dark brown, about 0.221 mm long and 0.198 mm broad, 8 or 9 inconspicuous ridges, outer colored membrane irregularly reticulate, size of reticulations about 0.01 mm across or larger, thick, opaque. (Only immature oospores seen in specimen described by Braun and Nordstedt.) ANTHERIDIUM: long stalked, up to 0.318 mm broad. Specimen seen: North Carolina: Wake co.; most abundant in the spring and autumn in the swift outflow from Partin's Mill Pond, water soft, about pH 5.5, clay bottom, 20 miles south of Raleigh, L. A. Whitford 3, May 24, 1941, April 19, 1946.

The Herbarium
Butler University

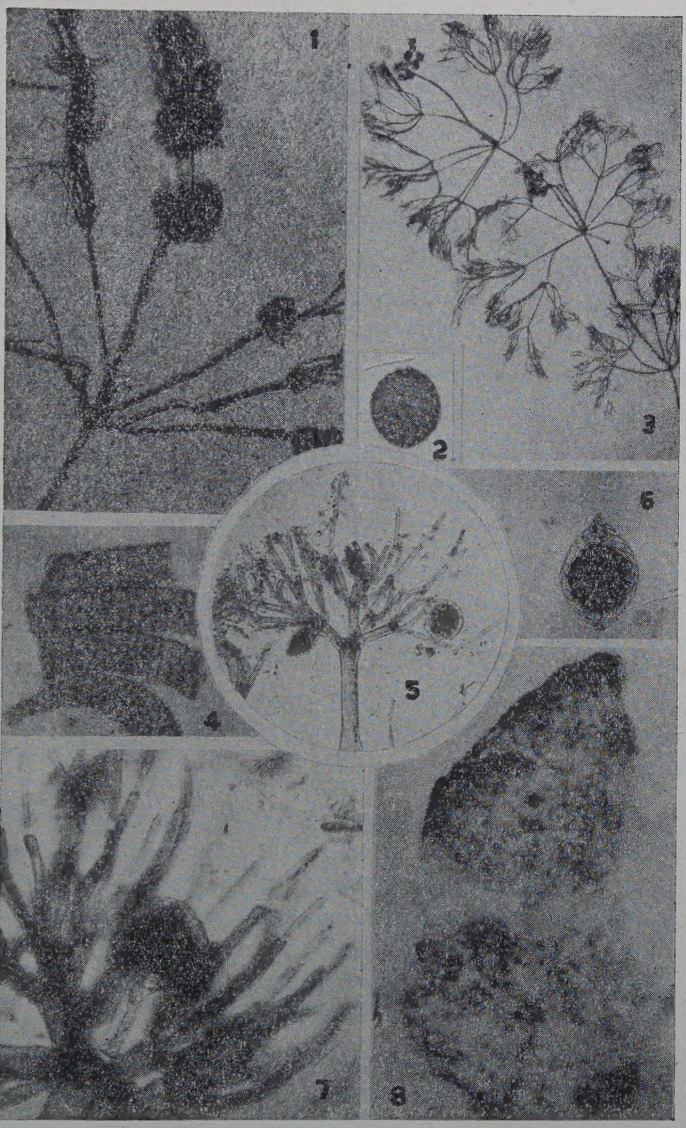


Plate I. NITELLA CAPILLATA A. Br. 1. Fruiting head. 2. Oospore. 3. Portion of plant. 4. Outer colored membrane of oospore. 5. Fertile branchlet. 6. Oogonium. 7. Portion of fertile branchlet greatly enlarged to show antheridium and ultimate rays. 8. Young fruiting head showing some internal structure. Figs. 1 and 8 photographed from dried material remoistened with water. Fig. 3 is of a dried herbarium specimen. Figs. 2 and 4-7 are from formalin preserved material.

FOSSIL DIATOMS FROM LAKEVILLE BOG, INDIANA[1]

By JACK R. WEAVER

In the study of diatoms, bogs furnish an excellent record of diatom flora during the post glacial period. As a result of the Late Wisconsin glacial activity Northern Indiana was dotted with numerous lakes, many of which are of the kettle-hole type and have become extinct due to deposition of marl and peat. In many bodies of water where diatoms are present, a portion of this sedimentation is composed of the silicified walls of the diatom cells which remain unaltered after the death of the organism. Thus diatoms become deposited and preserved in stratigraphic order.

Diatoms although a somewhat isolated group in the organic world, appear to be more sensitive indicators of ecological conditions than the higher plants. Diatom growth is controlled by such factors as temperature, light intensity, amount of dissolved gasses, minerals and nutrients as well as the chemical composition of the water. Most of these can become limiting factors thus influencing the flora present or preventing their growth entirely.

In bogs the water as a rule is high in humic acid and low in dissolved nutrients and oxygen. As a result, an acid condition develops which means that only a specialized flora can exist. The different species, which are commonly found in peat, have been referred to by Hanna (4) as the "peat bog flora." Some of the common diatom genera composing this flora are Eunotia, Pinnularia, Fragilaria, Navicularia, Melosira, Cyclotella, Nitzschia and Frustulia.

The study of Lakeville bog had two objectives first, to identify the species found at the various levels; and second, to use degree of abundance of certain genera and species as indicators of changing ecological conditions as the bog developed from an open lake to maturity.

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[1] A portion of a thesis submitted in partial fulfillment of the requirements for the Master of Science degree in the Division of Graduate Instruction.

HISTORY

Interest in peat bog profile studies first developed in Europe where their relation to ecological succession of plant associations was seen. The study of diatoms of bogs has been of a rather limited nature in this country. Hanna (4) reports that the flora which typically inhabits bogs was first made known in America by F. W. Lewis in 1864 and was determined by a study of peat material in the White Mountains of New England. Since that time there have been several workers who have made significant and important studies of the diatom floras in peat deposits. Among these as reported by Patrick (10) are Cunningham's study of a swamp deposit at Montgomery, Alabama, and Mann's study of diatom deposits found in an excavation at Washington, D. C. Also of importance are Hanna's (4) study of Florida peat deposits, Cocke, Lewis and Patrick's (3) study of Dismal Swamp peat and Patrick's (10) study of diatoms from Patschke bog, Texas.

Most of the studies by Americans except those of Dr. Patrick have been solely of a taxonomic nature. Dr. Patrick has shown that diatoms are sensitive indicators of their ecological habitats. By knowing these ecological relations, she has been able to determine the past climatic and water conditions associated with a bog from its origin until the completion of its deposition.

DESCRIPTION OF BOG

Lakeville bog is located in the north half of section 3 (35 north, 2 east), Union Township, St. Joseph County, Indiana. The bog had a total depth of 30 feet with water pockets at the 21- and 22-foot levels. The sediments are largely organic in nature except at the 29- and 30-foot levels which are composed of marl. The peat is dark brown in color, light in weight and obviously composed to a large extent of decayed vegetable debris.

Since glaciation was responsible for the formation of the bogs of Northern Indiana, it can be assumed that the history of Lakeville Bog begins early in post glacial times. It is obvious that in the formation of peat deposits the element of time enters in as an essential factor. Hanna (4) reports Soper and Osborn as computing the average rate of accumulation of peat for the Great Lakes region at the rate of .72-2.16 inches per century. By using these two rates of deposition it is seen that the time required for the filling of Lake-

ville Bog could vary from 16,000 to 50,000 years assuming that the rate of deposition had been constant. At best, these figures are only estimates and cannot be made with any claim of accuracy.

METHODS

The peat samples were obtained from Lakeville Bog by the use of the Hiller type borer. They were taken from each foot level from the surface to the bottom of the bog. This material was placed into bottles, corked and then sealed with paraffin pending its preparation for microscopic study.

The process of cleaning diatoms involves the separation of the small silicious frustules from the peat and other material composing the bog deposit. The cleaning method used in the present study was a rather simple procedure but produced excellent results. A small amount of peat material (2-3 cc.) was placed into a test tube with approximately 10 cc. of distilled water. The peat and water were then heated and violently shaken until all the peat material was thoroughly washed. The water and peat material were then placed into a small crucible and by the use of a pipette the water which contained the diatoms was removed, leaving most of the peat material in the crucible. This process permits the separation of a large portion of the diatoms present in the original sample.

A drop of water containing the diatoms was placed on a cover glass, distributed evenly over the surface and then dried. The prepared cover glasses were mounted on glass slides by the use of hyrax, a special mounting medium with a high refractive index.

Four slides with two cover glasses each were prepared for each foot level, thus assuring that most of the species present would be represented. Extreme care was taken to eliminate contamination of the material while in preparation, thus preventing introduction of errors in the record of distribution.

An attempt has been made to identify every species present in each foot level. In the 12- and 13-foot levels, where the flora is well developed, five hundred individual diatoms were identified and the percentage of each species observed was computed. The results of this computation are represented in table 1.

Reference to name authority, distribution and habitat characteristics (wherever possible) will be made in the systematic section of this paper.

OBSERVATIONS.

The distribution of diatoms in Lakeville Bog is rather unusual in that a well-developed diatom flora is expressed in only the 12- and 13-foot levels. Several of the remaining levels contain diatoms or numerous fragments, but these are not of sufficient abundance to be considered indicative of the conditions which were present at the time of deposition. Twenty species representing 11 genera of diatoms were observed to be present in the bog.

From the 30- to the 23-foot level there were no diatom frustules or fragments but numerous sponge spicules were observed. Sediments at the 22- and 21-foot levels were of an unconsolidated nature so that no sample could be taken. The 19- to the 16-foot levels contained a few scattered fragments of frustules which appeared to consist mainly of the genera Eunotia and Pinnularia. The 15-foot level had numerous species present but their frequency was very small. Very little material was available for observation in the 14-foot level, but a few frustules of *Gomphonema sarcophagus* and *Melosira italica* were observed.

In the 12- and 13-foot levels where the diatom flora is well developed the percentage of each species observed is represented in table 1. The 13-foot level had an abuandant flora with *Melosira italica* composing 94 per cent, *Cyclotella meneghiniana,* 3.4 per cent, and *Gomphonema sarcophagus,* 1.4 per cent. *Melosira italica* had the highest frequency and abundance of any species in the bog.

In the 12-foot level *Melosira italica* composed 55.4 per cent, *Cyclotella meneghiniana,* 11.2 per cent, and *Fragilaria construens* 9 per cent of the forms present. The larger species such as *Cymbella aspera, Cymbella cuspidata, Navicula americana, Navicula seman, Pinnularia major* and *Pinnularia streptoraphe* had their greatest abundance in the 12-foot level but in comparison with the smaller forms they do not appear very numerous. Most of the larger forms are present in the 13- and 15-foot levels but their abundance is very low.

All of the species present in the bog were found represented in the 12-foot level except *Amphora ovalis,* which was present only in the 13-foot level. All of the species tabulated had a greater abundance in the 12-foot level than in the 13-foot level with the exception of *Melosira italica.* From table I it is seen that the most abundant species appearing in the bog are *Melosira italica, Cyclotella meneghini-*

ana, Gomphonema sarcophagus and *Fragilaria construens.*

From the 11- to the 1-foot level there are only a few scattered fragments of diatom frustules with an occasional isolated form. *Cyclotella meneghiniana* appeared in the 7-, 6- and 5-foot levels with a very few frustules in each. The fragments present seemed to be mainly of the genera Eunotia and Pinnularia.

SYSTEMATIC SECTION

Amphora ovalis Kutzing 1844

Occurrence: only in the 13-foot level and represented by only three frustules. It is possible that they were carried into the bog by some external force. A fresh water species common in ponds.

Illustrations seen: Boyer (1) pl. 15, f. 7; Pasher (8) p. 142, F. 311; Van Heurck (7) pl. 1, f. 1-7.

Cyclotella meneghiniana Kutzing 1844

Occurrence: best represented in the 12- and 13-foot levels, although it appeared in the 6-, 7-, 14- and 15-foot levels. A common fresh water species.

Illustrations seen: Boyer (1) pl. 2, f. 6; Justedt (6) p. 100, f. 67c; Van Heurck (17) pl. 94, f. 11.

Cymbella aspera (Ehr.) Cleve 1894

Occurrence: in the 12-, 13- and 15-foot levels, being most abundant in the 12-foot level. Patrick (9) reports this species as being frequent in a lake with a pH of 7.3. A common fresh water species.

Illustrations seen: Boyer (1) pl. 18, f. 1; Hustedt (6) p. 365, f. 680; A. Schmidt (15) pl. 9, f. 2; Van Heurck (17) pl. 2, f. 8.

Cymbella cuspidata Kutzing 1844

Occurrence: in the 12-, 13- and 15-foot levels, being best developed in the 12-foot level. Frustules badly eroded or broken. A fresh water species generally distributed northward.

Illustrations seen: Boyer (1) pl. 18, f. 17; Van Heurck (17) pl. 2, f. 3; A. Schmidt (15) pl. 9, f. 50-53.

Eunotia formica Ehrenberg 1843

Occurrence: in only the 12- and 13-foot levels with only a few specimens represented. The length of the frustules seemed to vary somewhat. A fresh water species found in ponds of New England.

Illustrations seen: A. Schmidt (15) pl. 27, f. 305; Van Heurck (17) pl. 134, f. 1.

Eunotia monodon v. *major* (W. Smith) Hustedt apud Pasher 1930

Occurrence: most abundant in the 12- and 13-foot levels but also represented in the 5- and 15-foot levels. Frustules quite variable in size and striation. Patrick (9) reports this species as being in association with surface littoral vegetation. A fresh water species quite common in New England lakes.

Illustrations seen: A. Schmidt (15) pl. 287, f. 103; pl. 271, f. 13, 14.

Eunotia pectinalis v. *minor* (Kutz.) Rabenhorst 1864

Occurrence: most abundant in the 12- and 13-foot levels but also represented by one frustule in the 1-foot level. A fresh water species frequently found in swamps and bogs.

Illustrations seen: Hustedt (6) p. 80, f. 239; Van Heurck (17) pl. 33, F. 20-21.

Eunotia praerupta v. *bidens* (Ehr.) Grunow 1880

Occurrence: in only the 12- and 13-foot levels. Patrick reports it as being associated with Fontinalis and Sphagnum. A fresh water species common in peat deposits.

Illustrations seen: Boyer (10) pl. 13, f. 19; Hustedt (6) pl. 173, f. 213; A. Schmidt (15) pl. 273, f. 26-28; W. Smith (16) pl. 33, f. 284; Van Heurck (17) pl. 34, f. 20.

Fragilaria brevistriata Grunow apud Van Heurck 1881

Occurrence: in only the 12- and 13-foot levels with only a very few specimens present. A fresh water species common in lakes.

Illustrations seen: Hustedt (6) p. 145, f. 151; A. Schmidt (15) pl. 307, f. 10-14.

Fragilaria construens (Ehr.) Grunow 1862

Occurrence: only the 12-and 13-foot levels. The frustules present were extremely variable in outline. A common fresh water species.

Illustrations seen: Boyer (1) pl. 10, f. 30; Hustedt (6) p. 145, f. 131; W. Smith (16) 2: pl. 34, f. 291.

Gomphonema sarcophagus Gregory 1856

Occurrence: rather well developed in the 12- and 13-foot levels. A very few frustules were also present in the 14-, 15-, 17- and 18-foot levels. A fresh water species appearing as a fossil in Canada.

Illustrations seen: Boyer (1) pl. 19, f. 16; A. Schmidt (15) pl. 234, f. 36-37; Van Heurck (17) pl. 25, f. 2.

Melosira italica (Ehr.) Kutzing 1844

Occurrence: best expressed in the 12- and 13-foot levels although a few isolated frustules appeared in the 14-, 15-, 17- and 18-foot levels. As reported by W. Smith (16) there has been much confusion in the synomony of *Melosira italica, Melosira oricholea* and *Melosira italica* v. *crenulata.* W. Smith places all the above forms together because of the much diversity in the length and breadth as well as a greater or less distinctness of denticulations. Many spores of this species were present in the 12-foot level. A common fresh water species.

Illustrations seen: Van Heurck (17) pl. 88, f. 7.

Navicula americana Ehrenberg 1843

Occurrence: in the 12-, 13- and 15-foot levels. It apparently reached its best development in the 12-foot level where it was most common. A fresh water species and found occasionally in ponds.

Illustrations seen: Boyer (1) pl. 26, f. 8; Hustedt (6) p. 280, f. 464; Van Heurck (17) pl. 12, f. 37.

Navicula seman Ehrenberg 1843

Occurrence: in the 12-, 13- and 15-foot levels but reached its best development in the 12-foot level. Patrick (9) reports finding this species in a bog. A fresh water species found frequently as a fossil in the eastern states. It may occur in ponds of New England.

Illustrations seen: Boyer (1) pl. 26, f. 11; A. Schmidt (15) pl. 72, f. 1.

Nitzschia spectabilus v. *americana* Grunow, Cleve and Grunow 1880

Occurrence: apparently present in many levels of the bog; but because the frustules were always broken, it was difficult to make a determination accurately. It was apparently well developed in the 12- and 13-foot levels and was present in the 14- and 15-foot levels.

This form seems to be a fossil in post glacial deposits as reported by Boyer (1). A fresh water species present in New England ponds.

Illustrations: Boyer (1) pl. 33, f. 3; pl. 39, f. 1.

Pinnularia lata (Breb.) W. Smith 1853

Occurrence: in the 12- and 15-foot levels but had very few

frustules present in either one. A fresh water species present in New England.

Illustrations seen: Boyer (1) pl. 30, f. 23; A. Schmidt (15) pl. 45, f. 5-8; W. Smith (16) pl. 18, f. 167.

Pinnularia major (Kutz.) W. Smith 1853

Occurrence: rather well developed in the 12- and 13-foot levels; it appeared to be the most abundant species of Pinnularia present. A fresh water species common eastward.

Illustrations seen: Boyer (1) pl. 28, f. 4; A Schmidt (15) pl. 42, f. 8; W. Smith (16) pl. 18, f. 162; Van Heurck (17) pl. 5, f. 3-4.

Pinnularia streptoraphe Cleve 1891

Occurrence: rather well developed in the 12-foot level but also present in the 2-, 13- and 15-foot levels. A fresh water species present in New England ponds.

Illustrations seen: A. Schmidt (15) pl. 42, f. 7.

Pinnularia viridis Ehrenberg 1843

Occurrence: best represented in the 12-foot level but present, with only one frustule observed, in the 2- and 17-foot levels. A common fresh water species.

Illustrations seen: Boyer (1) pl. 29, f. 2; A. Schmidt (15) pl. 42, f. 11-14; Van Heurck (17) pl. 5, f. 5.

Tabellaria fenestrata (Lyng.) Kutzing 1844

Occurrence: most abundant in the 12- and 13-foot levels. Some fragments of this species were present in other levels but in no case did it become numerous. This species seems to prefer shallow water and is common in streams and ponds.

Illustrations seen: Boyer (1) pl. 8, f. 1; Brun (2) pl. 9, f. 13; Hustedt (6) p. 22, f. 99; W. Smith (16) 2: pl. 43, f. 317.

DISCUSSION

The distribution of diatoms in the Lakeville bog is rather unusual in that a well developed flora is present in only the 12-and 13-foot levels. The assemblage which is present, however, is very characteristic of peat bog floras. In order to give a detailed account of the successional change of the diatom flora in the bog, each foot level will be discussed as to the character of the sediments. The 30-foot level was composed of marl and contained little or no plant remains.

There were also no diatom frustules or fragments present in this level. At the 29-foot level sponge spicules were observed and these became increasingly more abundant to the 23-foot level where sponges apparently reached their maximum development in the bog. No diatom frustules or fragments were observed in any of these levels.

By comparing the ecological requirements of fresh water sponges as reported by Jewell (7) and those of diatoms, it is seen that they require many of the same conditions for existence. Especially important is the amount of dissolved salts and nutrients in the water. The fresh water sponges seem to have a wider range of tolerance to most ecological conditions such as nutrients, minerals, pH, and light than diatoms and therefore are able to exist where diatoms are not. In the levels where diatoms are present the number of sponge spicules seems to be less.

From the 20- to the 16-foot level conditions apparently were not favorable for the growth of either diatoms or sponges. Scattered fragments of diatom frustules were observed which were too small for positive identification, but appeared to consist mainly of the genera Eunotia and Pinnularia. These two genera are characteristic of strongly dystrophic water conditions.

Beginning with the 15-foot level conditions more conducive to the growth of diatoms must have been present. The flora of this level is rather well represented by numerous forms but all are very low in abundance. The available material from the 14-foot level was such a small quantity that the writer feels a true representation of this level was not obtained since very few frustules were observed of species which were rather abundant in the 15- and 13-foot levels.

The species present in the 15-foot level became most abundant in the 13- and 12-foot levels. In only these two levels is a diatom flora sufficiently abundant to indicate favorable environmental conditions. We must thus assume that the bog during the greater part of its history was not a favorable habitat for diatoms. The abundant planktonic forms in the 12- and 13-foot levels such as Melosira and Cyclotella seem to be closely correlated with the temperature of the water as well as with dissolved nutrients and gasses. Temperature can be one of the most important factors influencing diatom growth as it affects the solubility of salts present in the water.

The fossil pollen study of Lakeville Bog by Hamp (5) would indicate that warmer, drier climate was making an appearance about

the time the 15-foot level was deposited and continued during the remaining time of deposition of bog material. These conclusions are based on the assumption that an increase of Quercus indicates a warmer climate and a decrease of Acer indicates a drier climate. These conditions are noticeably apparent beginning at the 15-foot level, as indicated by the pollen frequency of these two species.

The diatom flora in the 11-foot level ends abruptly and does not appear again in any of the succeeding levels. The 11- to 1-foot levels have a few isolated forms which were present in the 12- and 13-foot levels but never reach a frequency of importance. The scattered fragments of diatom frustules in these levels would indicate that perhaps Pinnularia and Eunotia were the dominant species.

In summarizing the ecological factors which are indicated by the distribution of diatoms and the species present, it is evident that many changes took place during the filling in of the bog. The water conditions to the 23-foot level were perhaps of an oligotrophic nature, being low in dissolved nutrients, thus the absence of diatoms. From the 20- to the 16-foot level the water apparently became of a dystrophic character. At the 15-foot level the water became warmer and began to approach eutrophic conditions as evidenced by the large number of genera present. Apparently eutrophic conditions were reached in the 12- and 13-foot levels where the greatest distribution and frequency were reached. The abundance of such genera as Melosira, Cyclotella, Cymbella and Gomphonema are strongly indicative of eutrophic conditions. This condition was apparently short-lived as indicated by the sudden disappearance of the diatom flora from the 11- to the 1-foot level. The increase of such genera as Pinnularia, Eunotia and Gomphonema in the 12-foot level in comparison to the 13-foot level indicated perhaps a shallowing of the water which could bring about dystrophic conditions. This would account for the few scattered fragments of frustules of Eunotia and Pinnularia in the 11- to 1-foot levels which indicate dystrophic conditions.

SUMMARY AND CONCLUSIONS

1. This paper presents a study of fossil diatoms present in Lakeville Bog, Indiana.

2. Twenty species of diatoms representing eleven genera were found.

3. A well developed flora was expressed only at the 12- and 13-foot levels.

4. *Melosira italica* showed greatest abundance totaling 94.2 per cent of the organisms of the 13-foot level and 55.4 per cent in the 12-foot level.

5. The dominant species from the standpoint of abundance are *Melosira italica, Cyclotella meneghiniana, Gomphonema sarcophagus* and *Fragilaria construens.*

6. All species tabulated had a greater abundance in the 12-foot level than in the 13-foot level except *Melosira italica.*

7. Lack of diatom frustules or fragments in the 30- to the 23-foot level indicated that oligotrophic water conditions had existed in the bog when sediments of these levels were being deposited.

8. Warmer climate introducing eutrophic water conditions is perhaps responsible for the well developed flora in the 12- and 13-foot levels.

9. The scattered fragments of the genera Eunotia and Pinnularia in the 11- to the 1-foot levels suggest that dystrophic water conditions existed while sediments accumulated at these levels.

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TABLE I

Percentage of each species observed in a count of 500 diatom frustules from the 12- and 13-foot levels.

Species	12-foot	13-foot
1. Melosira italica	55.4 per cent	94.2 per cent
2. Cyclotella meneghiniana	11.2	3.4
3. Gomphonema sarcophagus	12.0	1.4
4. Fragilaria construens	9.0	.4
5. Cymbella aspera	3.2	
6. Eunotia monoden v. major	2.6	.6
7. Tabellaria fenestrata	1.8	
8. Navicula seman	1.4	
9. Eunotia formica	1.0	
10. Eunotia praerupta v. bidens	.8	
11. Navicula americana	.6	
12. Pinnularia streptoraphe	.4	
13. Pinnularia lata	.2	
14. Pinnularia major	.2	
15. Pinnularia viridis	.2	

TABLE II

Distribution of diatoms as to foot levels in Lakeville Bog.

Species	Foot levels 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19-30
1. Amphora ovalis													x						
2. Cyclotella meneghiniana						x	x					x	x	x	x				
3. Cymbella aspera												x	x		x				
4. Cymbella cuspidata												x	x		x				
5. Eunotia formica													x						
6. Eunotia monodon v. major					x							x	x						
7. Eunotia pectinalis v. minor	x											x	x						
8. Eunotia praerupta v. bidens												x	x						
9. Fragilaria brevistriata												x	x						
10. Fragilaria construens												x	x						
11. Gomphonema sarcophagus												x	x	x	x				
12. Melosira italica												x	x	x	x		x	x	
13. Navicula americana												x	x		x				
14. Navicula seman												x	x	x	x				
15. Nitzschia spectabilus v. american												x	x		x				
16. Pinnularia lata												x							
17. Pinnularia major												x	x						
18. Pinnularia streptoraphe		x										x	x		x				
19. Pinnularia viridis		x										x							
20. Tabellaria fenestrata												x	x						

A DIRECT MICROSCOPIC STUDY OF THE EFFECT OF TEMPERATURE ON BACTERIAL COUNT OF MILK[1]

By CHARLES F. HILL

INTRODUCTION

A bacterial count of milk is desirable to determine, in so far as it is possible, its quality. It has been thought by some workers that the direct microscopic method of counting the bacterial flora would give a complete history of the milk even after pasteurization. Others believe that heating so changes the bacterial cells that they cannot be detected in subsequent direct microscopic examinations of stained preparations.

Hastings and Davenport (3) report that many bacteria are changed when they undergo heating. They state that at pasteurization temperature the staining properties of bacterial cells vary considerably; some of the cells stain faintly, but evenly; some show decidedly uneven staining. Knaysi (4) and Ward (5) maintain that pasteurization not only kills bacteria commonly present in milk but renders them invisible when the film is stained with methylene blue. Kufferath, as reported by Hastings and Davenport (3), states that dead bacteria do not stain, but their presence is made evident by the fact that they appear in the film as clear areas in the background of stained casein, and that the number of bacteria present before heating can be obtained by noting the clear areas.

Lazarus (5) states that deeply stained cells in pasteurized milk indicate that they are thermoduric organisms.

Werner (7) reports that pasteurization has no effect on the staining properties of bacterial cells and that a direct microscopic count of pasteurized milk would show the same picture as would be shown in raw milk.

The purpose of this study is to determine by the direct microscopic method the effect of heat on the bacterial count of milk and to observe the fate of dead bacterial cells.

Since the above mentioned conflicts exist, this research is an

[1] A portion of a thesis submitted in partial fulfillment of the requirements for the Master of Science degree in the Division of Graduate Instruction.

attempt to determine by the direct microscopic method the effect of heat on the bacterial count of milk and to study the fate of dead bacterial cells.

METHODS

For the purpose of determining the effect of heat on the bacterial count of milk, determined by the direct microscopic method, ten specimens of market milks were used. These were designated as Specimen A, B, C, D, E, F, G, H, I, and J. It was found by experimentation that best results were obtained from specimens with high counts; therefore, the milk specimens were allowed to stand at room temperature for a period of from three to four hours before heating.

A quantity of the specimen was then put in each of seven sterile test tubes, stoppered, and numbered from one to seven. Sample No. 1 was used as a control and underwent no heating. Each of the other samples was heated in a separate waterbath with the temperature held constant for thirty minutes. The temperature range was as follows:

Sample No. 2..... 50°C for thirty minutes
Sample No. 3..... 60°C for thirty minutes
Sample No. 4..... 70°C for thirty minutes
Sample No. 5..... 80°C for thirty minutes
Sample No. 6..... 90°C for thirty minutes
Sample No. 7.....100°C for thirty minutes

The waterbath consisted of a 1000 cc. beaker of water held over a Bunsen burner by a ringstand. A centigrad thermometer, calibrated in degrees, was placed in the water. To maintain the desired temperature the burner was placed under the beaker of water or removed as the situation required. In this manner the temperature never varied over one-half a degree from the desired temperature.

Immediately after a sample had undergone the above treatment a microscope preparation was made. At the same time a microscope preparation was made of the control (Sample No. 1). These were labeled "series x." The test tube samples were then stored in a refrigerator at 5° Centigrade for twenty-four hours. At the end of this period another microscope preparation was made of the seven samples. These were labeled "series y."

The microscope preparations were made, as directed in Standard Methods (1), by using a sterile nichrome loop which was calibrated

to deliver 0.01 cubic centimeters of milk. This quantity was transferred to a clean slide, and spread evenly over an area of one square centimeter with a sterile nichrome needle. The preparations were then dried, placed in xylol for five minutes to remove the fat, dried again, and dipped in ninety-five per cent alcohol for five minutes to fix the material to the slide. After drying again, the slide was dipped in a saturated aqueous solution of methylene blue for two to five seconds. The slide was then rinsed in water and if overstained it was decolorized with ninety-five per cent alcohol (2).

A binocular microscope with 12.5X oculars and 1.8 immersion oil objective was used for counting the bacteria. This was adjusted to give a field with a diameter of 0.146 mm, representing 1/600,000 cubic centimeters of milk per field. Thirty fields of each sample in each series were observed and the number and types of bacteria were recorded.

In recording the types of organisms, diplococci were counted as one unit; the number of streptococccic chains were counted and recorded as well as numbers of cells making up the chains. Chains of six or more cells were considered to be long streptococci while those of five cells or less were considered to be short streptococci. Staphylococci and rods in clumps were counted and recorded both as individuals and as groups of organisms. In the tables accompanying this paper the streptococci, staphylococci, and rods in clumps have two figures listed for them. The first figure shown indicates the number of clumps; the other figure indicates the number of individual cells.

RESULTS

The results obtained by counting thirty fields of each specimen in each series are presented in table I.

TABLE I

Number of each type of organism per thirty microscopic fields as determined by direct microscopic count.

Specimen A "x series" *

Sample No.	Temp.	Dip.	Short Strep.	Long Strep.	Staph.	Isolated Cocci	Isolated Rods	Rods in Clumps
1.	**	7			2-10	44	88	12-72
2.	50°C	15	2-7	1-8		12	13	1-2
3.	60°C	23	6-27		1-6	7	19	5-14

TABLE I—(Continued)

Sample No.	Temp.	Dip.	Short Strep.	Long Strep.	Staph.	Isolated Cocci	Isolated Rods	Rods in Clumps
4.	70°C	26	2-8		1-6	8	22	
5.	80°C	7	1-3	1-10		7	12	
6.	90°C					5	9	
7.	100°C	5				5	15	
			Specimen A "y series" *					
1.	**	28	2-6	2-14	3-12	30	125	
2.	50°C	27	2-8	1-6	2-18	16	60	2-6
3.	60°C	12			1-5	13	28	1-13
4.	70°C	8				3	15	3-13
5.	80°C	6		1-10		5	10	
6.	90°C	1		1-6		4	2	
7.	100°C	4				4	5	
			Specimen B "x series" *					
1.	**	38				24	44	4-13
2.	50°C	5			1-8	8	27	2-7
3.	60°C	2				6	21	1-3
4.	70°C	3				3	16	1-3
5.	80°C	9				1	7	
6.	90°C	7				1	7	
7.	100°C	2				1	5	
			Specimen B "y series" *					
1.	**	50	1-4	1-12	1-12	43	55	23-163
2.	50°C	7		1-6		13	36	4-17
3.	60°C					3	17	1-9
4.	70°C	1				2	9	3-7
5.	80°C	1					4	
6.	90°C		1-4			1	6	
7.	100°C						3	
			Specimen C "x series" *					
1.	**	54	1-4			5	35	
2.	50°C	4				6	42	
3.	60°C	1				3	18	
4.	70°C	3					29	
5.	80°C	3				5	3	
6.	90°C	3					8	
7.	100°C	3					2	
			Specimen C "y series" *					
1.	**	1				2	144	
2.	50°C	6				4	36	
3.	60°C					6	21	

TABLE I—(Continued)

Sample No.	Temp.	Dip.	Short Strep.	Long Strep.	Staph.	Isolated Cocci	Isolated Rods	Rods in Clumps
4.	70°C	2				2	13	
5.	80°C	2				1	6	
6.	90°C						3	
7.	100°C	1						
			Specimen D "x series" *					
1.	**	139		2-33	5-81	9	201	43-776
2.	50°C	82		1-16	9-83	17	103	25-568
3.	60°C	38			10-62	10	177	4-26
4.	70°C	21		1-10	14-85	17	56	5-48
5.	80°C	25	1-5		16-88	19	25	
6.	90°C	28	5-20		18-75	13	9	
7.	100°C	23	5-19		10-58	8	7	
			Specimen D "y series" *					
1.	**	76	2-10	1-9	18-138	13	51	9-43
2.	50°C	23	2-8	1-8	7-65	10	65	1-12
3.	60°C	16	2-8		5-20	4	41	
4.	70°C	14	1-4		5-18	3	24	
5.	80°C	12			2-9	3	3	
6.	90°C	10			1-4	1	3	
7.	100°C	9					1	
			Specimen E "x series" *					
1.	**	145	2-7	3-31	7-82	11	179	40-790
2.	50°C	80		2-17	6-78	21	111	28-747
3.	60°C	37			10-68	12	171	5-28
4.	70°C	20		1-6	1-90	14	61	5-29
5.	80°C	20			17-86	18	28	
6.	90°C	19	2-17		13-67	13	11	
7.	100°C	19	2-9	1-8	10-52	9	8	
			Specimen E "y series" *					
1.	**	87	3-15	1-17	17-141	11	58	10-41
2.	50°C	39	3-12	1-7	7-73	12	54	2-7
3.	60°C	20	2-9	1-8	2-18	4	43	
4.	70°C	16	2-6		3-21	2	29	
5.	80°C	15			2-7	3	4	
6.	90°C	12			1-5		1	
7.	100°C	13					1	
1.	**	129		2-29	5-78	13	180	41-785
2.	50°C	79		1-12	8-78	15	116	38-496
3.	60°C	41			11-67	14	181	6-33
4.	70°C	19		2-13	10-93	19	48	6-41
5.	80°C	21	2-7		12-86	17		5-24

TABLE I—(Continued)

Sample No.	Temp.	Dip.	Short Strep.	Long Strep.	Staph.	Isolated Cocci	Isolated Rods	Rods in Clumps
			Specimen F "x series" *					
6.	90°C	25	3-12	1-9	14-76	10	12	
7.	100°C	21	2-8	2-16	10-59	5	7	
			Specimen F "y series" *					
1.	**	74	2-14	1-15	14-129	15	73	7-39
2.	50°C	25		5-40	9-57	17	61	2-9
3.	60°C	15		2-13	7-31	5	38	
4.	70°C	15	1-3		3-14		22	
5.	80°C	10			3-10		4	
6.	90°C	8			1-4	1		
7.	100°C	7					1	
			Specimen G "x series" *					
1.	**	126		3-26	5-81	7	183	44-787
2.	50°C	78	1-4	1-9	8-82	21	105	30-683
3.	60°C	43			10-67	11	171	4-24
4.	70°C	20	3-11		11-84	18	51	5-30
5.	80°C	22	2-8		10-76	21	27	
6.	90°C	23	3-12	1-7	9-63	15	12	
7.	100°C	19	2-8		11-56	7	12	
			Specimen G "y series" *					
1.	**	74	1-7	1-16	21-144	14	64	7-41
2.	50°C	25		3-21	8-74	9	67	3-17
3.	60°C	11	1-3	1-7	7-23	2	39	
4.	70°C	13			5-21	3	21	
5.	80°C	9			1-7	1	4	
6.	90°C	7			1-4	1	4	
7.	100°C	5					1	
			Specimen H "x series" *					
1.	**	141	1-4	2-22	6-94	11	189	34-781
2.	50°C	74	2-7	1-9	10-90	15	114	50-531
3.	60°C	40			8-60	9	117	5-23
4.	70°C	20		1-7	11-81	20	55	7-38
5.	80°C	26	2-7	1-13	12-79	17	24	
6.	90°C	26	3-12	2-17	13-76	12	10	
7.	100°C	19	1-4	1-7	9-55	6	6	
			Specimen H "y series" *					
1.	**	69	2-9	1-7	22-146	11	53	5-34
2.	50°C	36	2-9	1-11	9-71	12	47	2-9
3.	60°C	19	1-5		3-19	3	41	1-4
4.	70°C	15	1-4		3-24	1	27	

TABLE I—(Continued)

Sample No.	Temp.	Dip.	Short Strep.	Long Strep.	Staph.	Isolated Cocci	Isolated Rods	Rods in Clumps
5.	80°C	12	1-5		2-7	1	5	
6.	90°C	9				1	2	
7.	100°C	4					3	
			Specimen I "x series"*					
1.	**	126	2-7	3-32	4-70	11	195	29-822
2.	50°C	85		1-18	9-86	19	114	39-772
3.	60°C	40			9-60	9	117	5-26
4.	70°C	22	1-4	1-12	9-73	15	57	6-41
5.	80°C	28	1-4	1-11	16-95	22	21	
6.	90°C	23	2-9	1-7	14-72	10	10	
7.	100°C	21	1-3	2-14	11-61	6	8	
			Specimen I "y series"*					
1.	**	78	4-19	2-21	23-147	17	56	7-37
2.	50°C	29	2-10		7-74	11	54	2-14
3.	60°C	14	1-4	1-7	6-24	3	37	1-5
4.	70°C	15	1-4		6-21	2	21	
5.	80°C	11			1-7	1	4	
6.	90°C	7			1-4		4	
7.	100°C	5				1	1	
			Specimen J "x series"*					
1.	**	129		2-29	6-80	10	213	40-763
2.	50°C	85		1-14	16-98	18	116	35-741
3.	60°C	37			10-56	8	107	4-24
4.	70°C	23		1-6	10-74	15	59	6-34
5.	80°C	20	1-3	2-13	11-82	16	28	
6.	90°C	23	2-9	2-14	15-73	14	11	
7.	100°C	17	1-4	1-6	12-62	9	5	
			Specimen J "y series"*					
1.	**	72	3-14	1-9	17-147	11	61	9-47
2.	50°C	19	2-7	1-6	9-73	9	59	1-8
3.	60°C	14	1-4	1-6	4-24	3	43	1-4
4.	70°C	15			2-14	1	19	
5.	80°C	11			1-7		4	
6.	90°C	7				1	1	
7.	100°C	6					1	

*"x series"—slide specimens taken immediately after heating for thirty minutes at temperature indicated. "y series"—slide specimens taken after heating for thirty minutes at temperature indicated and being stored for twenty-four hours at 5°C.

** Sample No. 1 was used as a control and underwent no heating.

Throughout the process of counting numerous shadow forms were noted. The term "shadow form," described by Knaysi (4) is given to denote bacteria which did not take the methylene blue stain as deeply or as evenly as normal cells would. Hastings and Davenport (3), take this to be an indication of dead cells. Table II lists the number of fields in each sample of each specimen which showed these shadow forms:

TABLE II

Number of fields per sample of each specimen showing shadow forms

Temp.	A	B	C	D	E	F	G	H	I	J
					"x series"					
50°C	7	11	0	18	14	20	17	22	18	21
60°C	12	14	15	21	20	23	21	24	21	23
70°C	15	15	18	25	24	26	25	27	23	24
80°C	18	22	26	27	27	30	29	30	26	25
90°C	24	23	30	30	30	30	30	30	30	30
100°C	30	30	30	30	30	30	30	30	30	30
					"y series"					
50°C	4	17	8	16	14	19	21	15	17	14
60°C	7	20	10	30	30	30	30	30	30	30
70°C	12	22	16	30	30	30	30	30	30	30
80°C	14	26	30	30	30	30	30	30	30	30
90°C	21	27	30	30	30	30	30	30	30	30
100°C	30	29	30	30	30	30	30	30	30	30

The effect of heat on each specimen is shown in Table I. Specimen A shows the results of a direct microscopic count on a specimen of milk, with a low bacterial count, which has undergone heating for thirty minutes at the temperatures indicated. At a temperature as low as 50°C there was a definite decrease in the total number of organisms present. The decrease was irregular for each type of organism in "x series," but after storage for twenty-four hours, in "y series" the decrease was in more or less direct proportion to the temperature. From this it may be concluded that heating causes the cells to disintegrate and become invisible, but the rate of disintegration is irregular, causing some dead cells to appear as living cells. Only after twenty-four hours storage at a temperature low enough to prevent active reproduction may the effect of heating be demonstrated miscroscopically on stained specimens having a low bacterial

count. Specimens B and C (Table I) bear out the same results as Specimen A. Specimens A, B and C were all taken from market milks with low bacterial counts.

Specimen D (Table I) shows the results of a direct microscopic count on a specimen of milk, with a high bacterial count, which has undergone heating for thirty minutes at the temperatures indicated. This part of Table I shows that as temperature increased there was a fairly regular decrease in numbers of each type of organism in "x series" as well as in "y series." It is probable that the high count, resulting in better representation of each type of organism, caused this. In this specimen, as in the previous ones, the greatest decrease in total bacterial count as well as in total count for type of organisms present was in "y series."

Specimens E through J respectively, present the same picture. These are all the results of direct miscroscopic counts of stained preparations of milk with high bacterial counts. In all cases heating initiated a decrease in the total direct miscroscopic count of bacteria in milk.

In order to determine the overall effect of heat on the total count of bacteria in milk, Table III has been made showing the total number of visible organisms present in all specimens at the temperatures indicated and the percentages of decrease brought about by the given temperature.

TABLE III

Composite results obtained by totaling the microscopic counts of all specimens at temperature indicated.

Temp.	Total No. of Bacteria "x series"	Percent of Decrease	Total No. of Bacteria "y series"	Percent of Decrease
Unheated	9,075		3,194	
50°C	6,872	24.3%	1,630	40 %
60°C	2,164	76.2%	781	75.6%
70°C	1,687	82.2%	513	84 %
80°C	1,199	86.8%	221	93.1%
90°C	1,006	88.9%	128	96 %
100°C	789	91.3%	76	98 %

This evidence alone would refute the stand taken by Werner (7), who states that pasteurization has no effect on staining properties of bacterial cells. It is evident that the number of visible organisms

decreases with increased temperature. At 60°C, a temperature slightly below the usual pasteurization temperature (62.8°C) the total number of organisms dropped from 9,075 to 2,164 immediately in "x series," a decrease of 76.2%. After storage for twenty-four hours at 5°C, "y series," there was a drop from 3,194 to 781 visible organisms, a decrease of 75.6%.

In Table III it may be noted that the percentage of decrease by temperature was greater in "y series" than in "x series." It may be assumed that heating plus storing for twenty-four hours at 5°C caused the difference. It may further be noted that in "y series" the total percentage of organisms killed is 98% leaving only 2% alive or at least fully stained and visible. Hastings and Davenport (3) report a 3% minimum for milk pasteurized at 62.8°C for thirty minutes.

By totaling the counts of each type of organism in all specimens of the same series, Table IV shows the effect of heat on types of organisms commonly found in milk.

TABLE IV

Composite results of total counts of each type of organism in all specimens.

Sample No.	Temp.	Dip.	Short Strep.	Long Strep.	Staph.	Isolated Cocci	Isolated Rods	Rods in Clumps
				"x series"				
1.		1034	6-22	17-202	42-671	145	1508	347-5589
2.	50°C	584	5-18	9-103	67-603	152	861	248-4547
3.	60°C	282	6-27	0	69-446	89	1099	39-201
4.	70°C	177	6-23	7-54	67-586	129	454	41-264
5.	80°C	181	10-37	5-47	94-592	143	175	5-24
6.	90°C	177	20-81	7-54	96-502	93	99	0
7.	100°C	149	14-51	7-51	64-403	56	75	0
				"y series"				
1.		609	20-98	11-120	136-1016	167	741	77-426
2.	50°C	236	13-54	14-105	58-505	113	539	19-99
3.	60°C	121	9-39	6-41	35-174	46	348	6-28
4.	70°C	114	6-21	0	27-133	19	200	9-26
5.	80°C	88	1-5	1-10	12-54	15	48	0
6.	90°C	61	1-4	1-6	5-21	10	26	0
7.	100°C	54	0	0	0	5	17	0

In general it can be noted that the decrease in number of each type of bacteria was much more regular in "y series" than in "x

series." This is probably due to the effect of time combined with the low temperature during the period of storage. Considering autolysis to be the cause of the decrease in numbers, this explanation is logical.

The time period between "x series" and "y series" was twenty-four hours. This would allow sufficient time for the cells killed by heat to disintegrate and, due to the low temperature, reproduction would be at a minimum. The result would be fewer visible cells.

In "x series" the total number of type organisms involving groups of cells was irregular in decrease although the numbers of cells per group was reasonably even or regular in decrease. (See Table V.)

TABLE V

Average number of cells per group.

"x series"

Temp.	Short Strep.	Long Strep.	Staph.	Rods in Clumps
Unheated	3.3	11.9	16.0	16.1
50°C	3.6	11.4	9.0	18.3
60°C	4.5	0	6.5	5.2
70°C	3.8	7.7	8.7	6.4
80°C	3.7	9.4	6.3	4.8
90°C	4.0	7.7	5.2	0
100°C	3.8	7.3	6.2	0
		"y series"		
Unheated	4.6	11.0	7.4	5.5
50°C	4.2	7.5	8.7	5.2
60°C	4.3	6.8	4.7	4.6
70°C	3.5	0	4.9	2.8
80°C	5.0	10.	4.5	0
90°C	4.0	6	4.2	0
100°C	0	0	0	0

It may be noted in Table V that the average number of rods in clumps "x series" at 60°C was 5.2, a drop from 18.3 for 50°C. In Table IV the total number of isolated rods at 60°C was 1099 which was an increase as compared to the same type organism recorded for 50°C. It is probable that the heating process killed and rendered invisible many of the rods in clumps causing the remainder to appear as isolated rods. This would decrease the total number of rods in

clumps and increase the number of isolated rods. The same explanation would account for the decrease in long streptococci at 60°C.

Throughout the direct microscopic examinations of the prepared films numerous shadow forms were observed. It was interesting to note that only rod shaped shadow forms were observed. There was no evidence of a coccus shape organism producing a shadow form. This may be due to confusion of coccus shadow forms with artifacts in the film.

It was noted that the number of fields showing shadow forms increased with increased temperature. It was also noted that in "y series" the temperature at which all fields showed shadow forms was lower than in "x series." (See Table II.)

There was no correlation between total numbers of all cells killed or total number of rod forms killed by heating and the number of shadow forms present. Table VI shows the results of an attempt to correlate the number of shadow forms present with the total number of cells killed and also with the total number of rod forms killed.

The number of rod forms killed by heating was determined by the difference between the total number of rod forms present in Sample No. 1 and sample heated to temperature indicated. The same procedure was used in determining the total cells killed with the exception that the totals of all types of organisms were used. The number of shadow forms was determined by actual count. Specimens D and E are presented.

TABLE VI

Comparison of the number of shadow forms present and the number of rods and total cells killed.

Specimen D

"x series"

Temp.	Total No. of Cells Killed	Total No. of Rods Killed	Total No. of Shadow Forms	Rods Unaccounted For	Total Cells Unaccounted For
50°C	370	306	97	209	273
60°C	926	774	94	680	832
70°C	1002	872	86	786	916
80°C	1077	952	91	861	986
90°C	1094	968	83	885	1011
100°C	1124	970	76	894	1048

TABLE VI—(Continued)

"y series"

Temp.	Total No. of Cells Killed	Total No. of Rods Killed	Total No. of Shadow Forms	Rods Unaccounted For	Total Cells Unaccounted For
50°C	1048	900	103	797	945
60°C	1150	936	141	795	1009
70°C	1176	953	109	844	1067
80°C	1212	974	87	887	1125
90°C	1221	974	59	915	1162
100°C	1229	976	42	934	1187
		Specimen E			
		"x series"			
50°C	191	111	109	2	82
60°C	929	790	94	686	835
70°C	1025	877	98	779	927
80°C	1093	939	93	846	1000
90°C	1128	956	82	874	1046
100°C	1140	959	53	906	1087
		"y series"			
50°C	1041	908	97	811	944
60°C	1143	926	169	757	974
70°C	1174	940	144	795	1030
80°C	1216	965	125	842	1083
90°C	1227	968	103	865	1124
100°C	1229	968	57	911	1162

From the above data it is evident that there is no correlation between the number of shadow forms and the number of rods killed or the total number of cells killed. This is in agreement with Knaysi (4) and Ward (6) and in disagreement with Werner (7) and Kufferath, as reported by Hastings and Davenport (3). Heating appears to render the organisms invisible when stained with methylene blue rather than causing most of them to show as shadow forms.

It was noted that the average number of shadow forms decreased with increase in temperature. It is probable that the rate of autolysis is increased with increased temperature; thus the organism would become invisible more quickly as temperature increased. There were no clear areas noted in the films that would indicate dead cells.

CONCLUSIONS

1. The number of visible organisms in milk decreases as temperature increases.

2. Heating followed by storage at 5°C for twenty-four hours further decreases the total number of visible organisms in milk.

3. Autolysis of dead cells increases with increase in time and temperature.

4. Shadow forms are present in milk that has undergone heating for thirty minutes at 50°C or higher.

5. The number of shadow forms in milk, heated from 50°C to 100°C for thirty minutes, decreases as the temperature increases.

6. There is no correlation between the number of shadow forms present and the number of bacterial cells killed.

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. C. Mervin Palmer for suggesting this problem and for his direction and guidance in completing this work.

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PROGRESS IN SUCCESSION IN THE PENNINGTON GROVE OF PINUS VIRGINIANA[1]

By J. E. Potzger and Esther Whitney Potzger

In 1932 the senior author[2] reported on an ecological survey in stands of *Pinus virginiana* in Monroe County, Indiana, viz. the Pennington grove near Weimer Lake, two miles sw. of Bloomington, on highway 45, and the Christie-Freeman groves, four miles nw. of Ellettsville. At the time of the 1931 survey very little ground cover had developed in either of these groves. The present study considered only the Pennington grove. This stand has seen no decided cultural modifications since the 1931 survey. Periodic surveys were made in the stand by various groups of ecology students of Butler University. These surveys showed a definite trend in succession which now definitely forecasts replacement of the 100 per cent coverage of crown by *Pinus virginiana*. The invaders consist of a large number of broadleaved species (table I). The forest floor, which in 1931 was almost bare, is today extensively covered by herbs and the seedlings of tree and shrub species. The authors made a survey in July and September of 1947 to define in quantitative terms the trends in succession in the Pennington grove.

We express sincere thanks to Mr. J. C. Pennington for permission to carry out the study on his property.

METHODS

The tree population was studied by aid of twenty 100-square-meter quadrats, each sample plot alternated with a skip of ten meters. All stems one inch or above in DBH. were measured with calipers and then tabulated for abundance data; all stems less than one inch DBH., but at least one meter in height, were tabulated (table I). Dead stems of Pinus and Juniperus were measured for diameter and then tabulated (table IV). The seedlings of woody plants were

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[1] This is contribution 196 from the botanical laboratory of Butler University, Indianapolis, Indiana.

[2] Potzger, J. E. Some observations on *Pinus virginiana* Mill. in Monroe County, Indiana: An ecological study. Indiana Acad. Sci. Proc. 41:153-174. 1932.

studied in 50 one-meter quadrats. Tabulation was made to determine, successional trends in the young stem size-class (table II). To show the changes in the vegetation over a period of years, results of surveys made in 1935 are compared with results obtained in 1947 (table III).

RESULTS

Table I presents the quantitative data of the 1947 survey in the Pennington grove. *Pinus virginiana* (total of 251 stems) with a F. I. of 100 per cent has at present no competitor in the large-stem sizes. The stand is still typical Pinus consocies, but *Quercus velutina* (F. I. 100 per cent), *Carya cordiformis* (F. I. 50 per cent), Sassafrass (F. I. 65 per cent), *Fagus grandifolia* (F. I. 55 per cent), Fraxinus (F. I. 85 per cent) forecast severe competition for the pine in the decade ahead. In fact, this competition may be active even now. *Pinus virginiana* shows little representation in the small-stem sizes, seedlings apparently persist only for a year or two. Table II reflects the same general conditions as table I, i. e., Pinus fails to maintain itself by adequate reproduction, and numerous broadleaved species, specifically *Quercus velutina,* successfully invade and reproduce under the open crown cover of the Pinus consocies. The important representatives in the sapling and young tree stages (table I) are *Quercus velutina,* Fraxinus, *Carya cordiformis, Fagus,* Sassafrass. *Cornus florida* is a significant competitor in early succession. Most of these same competing species also play the important role in the seedling layer (table II). The abundance of seedlings of *Pinus virginiana* (table II) and absence of this species in the small-tree sizes picture the mortality of this intolerant tree. How this elimination involves even quite old trees is shown in table IV. As shown there, on an average, two dead stems appear in every 100-square-meter quadrat.

The comparison of the 1935 and 1947 surveys shows significant changes in the stand, especially with respect to control exerted by *Pinus virginiana.* There is a decided decrease in the percentage of trees in the small-stem sizes, excellent survival of stems which represent the crown cover, but most significant is the increase in the number of species participating in the association complex (table III).

DISCUSSION

The Pennington grove began its expansion into eroded, wornout soil adjacent to the Cree homestead about 1885; its migration was from three trees which stood near the house. The last of these parent trees died in the late 30's of this century. The invading, aggressive pine developed an almost undisputed crown cover, but now seems to yield to a group of more tolerant invaders, which not only show aggressive reproduction under the crowns of the pines but are already well established in the youthful stem-size groups. There is, however, every indication that *Pinus virginiana* was needed to prepare the soil for such invasion. It is the most aggressive invasion, ecesis and competition which the authors have observed anywhere. Apparently it required 60 years to build up better edaphic conditions, especially better water-holding capacity of the soil, to enable Quercus, Carya, Fraxinus, and even Acer and Fagus, to find suitable habitat conditions. While replacement of Pinus is almost certain, the modus operandi of this succession will be a slow process of gradual replacement of pine as death, year by year, reduces its abundance in the stand. It may require another quarter, or perhaps even half century, to complete succession; however, it is definitely in progress now.

A typical mixed mesophytic forest cover, in which Quercus (primarily *Q. velutina*), Carya, Fraxinus, Acer and Fagus will be important representatives, is indicated. A second layer tree stratum is developing now (Ostrya, Cornus, Cercis, Asimina). The pioneers in the shrub and liana stratum (*Viburnum prunifolium,* Vitis, Celastrus, Parthenocissus, and *Rhus radicans*) are already a typical assemblage of those present in the Indiana mixed mesophytic forest. Since reproduction by *Pinus virginiana* has ceased, succession will without doubt be more rapid in the coming 15 years than it was in the past 15 years. This study emphasizes excellent qualities of the Jersey pine as a cover tree on soil which is too poor for successful immediate secondary succession by broadleaved species. Because the pine can successfully establish itself in eroded upland areas it can well be used throughout southern Indiana as initial forest cover on poor soil. After edaphic conditions have improved under protection of such pine stands broadleaved species, especially oak and hickory, will by natural succession invade and eventually replace them.

SUMMARY AND CONCLUSIONS

1. The paper deals with successional features over a period of 15 years in the Pennington grove.

2. In 1932 the stand represented a consocies of *Pinus virginiana* in which broadleaved species (trees, shrubs, lianas) were absent even in the ground layer.

3. Representation of *Pinus virginiana* has increased in the size-classes of 3 to 20 inches DBH. but has declined significantly in the 1- to 3-inch size classes.

4. Mortality rate is high for the pine, especially in the seedling to 2-inch size classes. On an average two dead stems occur in every 100-square-meter quadrat.

5. Great abundance and high F. I. are expressed by Quercus, *Carya cordiformis,* Fraxinus, Sassafras, and to a lesser extent by *Fagus grandifolia.*

6. While no immediate elimination is indicated, *Pinus virginiana* will definitely be replaced by broadleaved species because of lack of reproduction. Each dead tree of the pine is being replaced by aggressive, more tolerant broadleaved species.

7. In 1935 woody species in the forest complex consisted of 8 tall tree species, 2 small tree species and 2 lianas. In 1947 there were 19 tall tree species, 6 small tree species, 5 shrubs, and 2 lianas.

8. It is suggested that *Pinus virginiana* should be excellently adapted for cover tree in areas of badly eroded and wornout land in southern Indiana. It has the characteristic to form dense consocies until improved soil, which develops under its protecting crown cover, permits Quercus and Carya, as well as other broadleaved species, to invade the stand and eventually replace the pine.

TABLE I

Results of tabulation in 20 quadrats (100-square-meters each) in the Pennington grove of *Pinus virginiana*, showing distribution of size-classes and F. I. of woody species. Sizes in inches.

Species	Below 1 in.	1 in.	2	3-5	6-8	9-10	11-15	16-20	Above 20	Total above 1 in.	Percent F.I.
Acer rubrum	1										5
Acer saccharum	1										5
Asimina triloba	1	2								2	10
Amelanchier canadensis	2										5
Carya cordiformis	27										50
Carya laciniosa	1										5
Carya ovata	2										10
Carpinus caroliniana	1										5
Cornus florida	282	7		7						14	100
Corylus americana	1										5
Crataegus sp. ?	1										5
Fagus grandifolia	17										55
Fraxinus sp. ?	68		1							1	85
Juglans nigra	9	1								1	40
Juniperus virginiana	5										25
Lindera benzoin	4										15
Morus rubra	1										5
Nyssa sylvatica	1										5
Ostrya virginiana	2	1									15
Pinus virginiana		1	4	33	44	35	29	5		251	100
Platanus occidentalis		1	1	1						2	15

TABLE I—(Continued)

Species	Below 1 in.	1 in.	2	3-5	6-8	9-10	11-15	16-20	Above 20	Total above 1 in.	Percent F.I.
Prunus serotina	2					1					10
Quercus alba	1										5
Quercus borealis var. maxima	2										10
Q. velutina	188	2								2	100
Ribes sp. ?	5										5
Sassafras albidum	41	4			1					5	65
Ulmus americana	7			1						1	25
Ulmus fulva	1										5
Viburnum prunifolium	3										10
Vitis sp. ?	1										5

TABLE II

Abundance and F. I. in 50 one-meter quadrats. Pennington grove. Showing seedlings of woody plants.

Species	Number stems	Percent F. I.
Acer saccharum	25	16
Asimina triloba	1	2
Carya cordiformis	12	22
Cercis canadensis	3	6
Cornus florida	41	38
Fagus grandifolia	4	4
Fraxinus sp. ?	21	16
Juniperus virginiana	14	22
Liriodendron tulipifera	1	2
Morus rubra	1	2
Ostrya virginiana	2	4
Pinus virginiana	42	44
Prunus serotina	31	17
Quercus velutina	36	52
Sassafras albidum	42	44
Ulmus americana	14	26
Celastrus scandens	9	14
Parthenocissus quinquefolia	24	32
Rhus radicans	267	84
Rubus sp. ?	58	50
Viburnum prunifolium	4	6
Vitis sp. ?	11	14

TABLE III

Comparison of results obtained in 1935 and 1947. Showing size-class distribution of Pinus virginiana as percent of total number of stems and number of woody species participating in the association complex.

Stems of pine in inches DBH.	1935	1947
1 inch	7.0	2.2
2 inches	14.0	6.3
3-5	36.0	40.0
6-8	29.0	32.0
9-10	8.0	13.6
11-15	3.0	5.4
16-20	1.5	2.0
Number of tall tree species	8	19
Number of small tree species	2	6
Number of shrub species	–	5
Number of species of lianas	2	2

TABLE IV

Number of dead stems of Pinus and Juniperus in twenty 100-square-meter quadrats. Diameter of stems in inches.

Species	Below 1 in.	1	2	3	4	5	6	9	Total
Juniperus virginiana	3								3
Pinus virginiana	–	6	18	6	3	1	4	1	39

A POLLEN STUDY IN THE TENSION ZONE OF LOWER MICHIGAN[1]

By J. E. Potzger

A transition zone between two large vegetation cover types always presents a tantalizing aspect of vegetational characteristics, frequently suggesting instability. In such areas the microclimate of edaphic factors favors first one and then another group of species which are characteristic components of one or the other of the flanking formations, or smaller fluctuations in temperature and moisture find expression in vegetational changes which will not be evident within the boundaries of the definitely expressed formations. This is emphasized boldly by disjunct distribution or intermingling of species of these flanking climaxes, where habitat exerts selective action far greater than is possible under optimum climax control; this may at times even dim the "real climax" status. As Dachnowski (3) points out, pollen profiles can here, perhaps, give the most reliable picture of the climatically favored vegetation. It is, therefore, also logical to expect that small variations, or fluctuations, in climate ought to be recorded first in such tension areas by minor changes in vegetation, especially by variation in degree of importance of certain genera in the association complex.

It hardly requires deep botanical knowledge to note as one travels northward from Indiana to the upper half of Lower Michigan that soon after crossing a line marked roughly by highway 46, running approximately northeast to southwest from Saginaw to Muskegon, Michigan (fig. 1) that forest composition gradually changes by intrusion of more and more coniferous species into the broadleaved forest complex. During the decades which marked the close of the last century and the beginning of the present one, north of the beforementioned line began the great lumbering activities in the pine forests of Michigan. One naturally wonders why this sharp control existed, and how long it had prevailed. So it was planned to make pollen

[1] This is contribution 192 from the botanical laboratories of Butler University. The field work was supported by a research grant from the AAAS through the Indiana Academy of Science. Sincere thanks is expressed for this aid.

analyses of a series of bogs located along highway 46, and to include also some bogs which are located within 30 miles to the north and the south of the tension line. This 60-mile wide zone, bounded on the south by Allegan County and on the north by Oceana and Clare counties, gave promise of throwing some light on the forest development in the tension area since post-glacial times.

Thanks is expressed to the owners of the various tracts of land where the bogs are located for friendly co-operation and ready permission granted to carry on the work on their property; to my brother Gerhard and to my nephew Elmer Tessin for aid given with the boring operations; to the State of Michigan Department of Conservation, Geological Survey Division for the outline map used as basis for figure 1.

CHARACTERISTICS OF BOGS IN THE AREA

It is perhaps essential to discuss the bogs involved in this study somewhat in general because of certain unusual features associated with them. The author was very familiar with the topography of the counties bordering on Saginaw Bay, however, he had no experience with profiles of bogs of the region. Many lowland areas here are covered by typical bog vegetation, and so, externally, look like the bogs to the north and to the interior of Michigan. It was a distinct surprise, therefore, that the peat overlying sand was so extremely shallow in all of Saginaw and Midland counties, at times not exceeding six inches. As this study shows, not a single bog in Saginaw County yielded sufficient peat to assure a confidence-inspiring pollen analysis. Even in the adjoining counties peat deposits were as a rule still very shallow. It appeared to the writer that the numerous dry kettle holes one encounters west of Saginaw County indicate lack of sufficient bank and bottom sealing to retain water. This is even true in part for such bogs as Mud Lake, Meeuwsen Farm, Berens Bog and the deep Powell Bog whose basins were, perhaps, never filled to the top with water. In the Muskegon area numerous lakes which have become decadent during the last two decades because of decrease of water, suggest the same poorly sealed, reservoir. Included in this category would be Blue Lake, Mud Lake and a number of others where borings disclosed only a shallow accumulation of organic matter which rested on sand.

The Pleistocene history of the region offers an explanation for shallow basins and absence of peat, especially in the Saginaw area. According to Leverett and Taylor (6) the present-day Saginaw and Midland counties are part of an old lake plain of glacial lakes Saginaw and Warren which extended westward to near St. Louis, Michigan. No depressions of any consequence were formed. As a whole, therefore, the whole topography is generally flat and the interdunal marshes, or rather bog-like areas, with typical bog vegetation, did not retain sufficient water to favor accumulation of abundant organic matter. During rainless seasons such areas may always have been quite dry. Vegetation from adjacent locations must, thus, have moved into Saginaw and Midland counties as the waters of lakes Saginaw and Warren receded. On the other hand, forest succession could not have progressed very far previous to recession, for the Dow Marsh (figs. 1, 2) records the Picea period as well as the bogs farther west in the state do.

DESCRIPTIONS OF BOGS STUDIED

Meeuwsen Farm. This large, rather deep-set, bog is at present under cultivation, used to raise celery, mint and various other garden crops. A ditch drains at least to a depth of six feet, which probably accounts for the sparse pollen representation in upper foot-levels. It is located about 15 miles east of Holland, Michigan, along the Filmore-Dorr road in upper Allegan County.

Berens Bog. This bog is located on the farm of Mr. John C. Berens, about one mile sw. of the Meeuwsen farm. The extremely deep kettle hole with 50-foot high slopes has a shallow peat deposit of only five feet. At times it is entirely dry, but during wet seasons it presents the aspect of a lily pond.

Duck Lake Bog. This small bog comprises about two or three acres in a pasture along highway C-555, one mile southeast of Duck Lake, Crystal twsp., Montcalm County. The surface has been influenced by fire. Peat and lake sediments totalled ten feet.

Redman Mint Farm. During the early history of Gratiot County a low, wet tamarack swamp occupied several miles a short distance west of Ithaca in Newark twsp., sec. 3. Ditches drain the muck land, and it is at present laid out in extensive mint farms. Peat totals only four feet in depth. It is a difficult matter to locate the deepest part of a depression in such an extensive area, especially since the central

portion may not always be the deepest part. The boring was made on the farm of Mr. Ernest Redman in or near the geographical central part.

Dow Marsh. The wet sedge-meadow is flanked by sandy uplands and represents the best peat deposit found in Midland County. It comprises about ten acres, and it is located on the east side of highway 10, a quarter of a mile southeast of the junction between highways 10 and 20. The land is the property of the Dow Chemical Company of Midland, Michigan. Peat totalled four feet.

Carroll Farm. The peat deposit on the farm of Mr. Carroll is located in a shallow basin which gives the impression of having de-

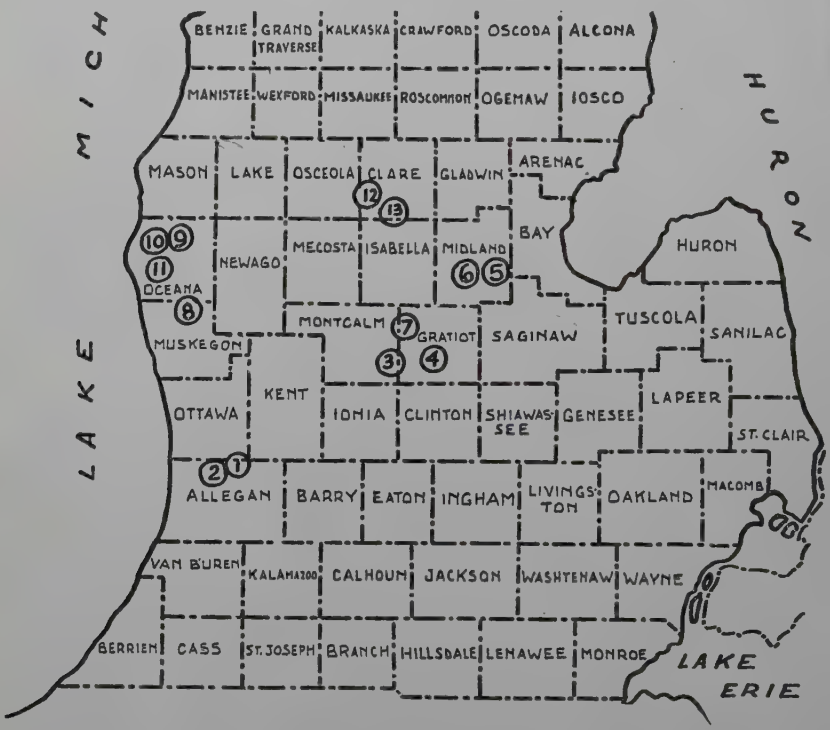


Fig. 1. Map of Central Michigan showing location of bogs studied.

veloped under semi-stream valley conditions. Peat totalled four feet. It is located five miles west of Midland, Midland County, on highway 20.

Austin Bog. This beautifully circumscribed bog is located along

highway 46 on the property of Mr. Alfred Austin, 10.5 miles west of St. Louis, Gratiot County (R.4, T.12, Sec. 20). It comprises an area of about 10 acres.

Blue Lake Bog. About a quarter of a mile north of the nearly extinct Blue Lake, Muskegon County, is a bog in early shrub stage of succession, comprising about 5 acres. It is 8 miles nw. of the little town of Twin Lake on highway 20, located on the west side of the country road. The approximate physiographic center of the bog yielded a 14-foot-deep core. Preliminary examination of the peat showed the Picea period lacking. A more careful survey on a second visit disclosed a basin which sloped towards the northern border of the bank. Here 18.5 feet were encountered. In the lowest levels of this second core the Picea period was well expressed. A forest of young oaks (about 75 per cent of the crown cover) with scattered *Pinus strobus* intervened as a 500-foot wide strip between the bog and the road.

Mud Lake. The almost decadent Mud Lake is located two miles east and one mile north of Shelby, Oceana County. It, too, is an extremely deep kettle hole with poorly sealed retaining walls of sand and gravel; hence, only the deepest part of the kettle was filled with lake sediments and peat. The western portion is still a muddy pond with shallow water. The boring was made at the very edge of the pond where peat supported weight of the worker only on hummocks of sedges. Preliminary borings disclosed that the slope of the bottom was towards the ponded area. Very likely, several feet of additional depth to that shown in the core taken is to be expected in the center of the muddy pond, though, the boring penetrated the extremely well-defined *Picea glauca* period for a depth of four foot-levels. Thus the whole history of the forest is represented in the core obtained.

Schroeder Bog. The author has repeatedly pointed out that bogs with central open ponds as a rule yield disappointing incomplete pollen profiles. This was also the experience with the beautiful Schroeder Bog, located about two miles west of Shelby, Oceana County. Most of the elongated bog is well solidified, and is covered by dense growth of relic *Picea mariana, Tsuga canadensis* and tall bog shrubs. The deepest place in the basin is no doubt covered by the 150-foot wide pond, and even though the boring was made at the very edge of the pond, the Picea period was wanting in the core.

The profile was included in this report (fig. 3) to show the later succession, especially as to correlation with changes recorded in the nearby Mud Lake profile (fig. 3).

Shelby Bog. The Shelby Bog represents one section of an irregularly-shaped chain of valley depressions. In one part of the basin a two-foot deep layer of marl has been removed. The bog is located on the property of Edward V. Babinec, on the west side of highway 31, at the very southern limits of Shelby.

Powell Bog. Most of the bogs in central Michigan are comparatively shallow, but the Powell Bog is a striking deviation from the rule. Topographically it represents a magnificent, almost circular, kettle, flanked by 25- to 50-foot high slopes which terminate in sandy uplands. Peat totalled 50 feet in depth (fig. 3). The central portion of the surface is still in Sphagnum and low shrub cover, indicating a rather recent "Verlandung." The border belt is covered with forest composed of young *Picea mariana,* Larix and various deciduous shrubs and trees. Results of trial borings suggest that from a gentle slope the kettle suddenly drops into the deep central portion, which alone had a pollen profile recording a decided *Picea glauca* dominance in the initial forest cover (fig. 3). Its location is five miles nw. of Farwell, Clare County, on highway 10, and one mile north of the highway, with pastured lands intervening. The property belongs to Mr. Rufus Powell.

Farwell Bog. Immediately east of Farwell, Clare County, highway 10 bisects a small bog of several acres extent. It is at present covered by one-foot-deep water. The depression is well isolated by encircling low ridges. Several trial borings indicated that the deepest part of the basin is about 25 feet north of the highway. Here the samples were taken.

METHODS

Peat samples were taken with a Hiller-type borer. The outer layer of the core within the cylinder was cut off with a knife and the sample was secured from the central part of the core. The samples were placed into one-ounce bottles, and these were securely stoppered. A label indicated location and foot-level. As a rule, samples were taken at one-foot intervals, but in some instances, where sharply differentiated layers appeared in the sediments, samples were taken at closer intervals. The laboratory technique followed closely the

Geisler (4) method; staining was with 5% aqueous solution of Gentian violet. Coarse, raw peat was separated with 95% alcohol and then strained through cheesecloth to remove the large organic particles. Tabulation was carried out under 150X magnification, and critical examination of all small and not easily recognized pollen grains, as well as measurements of all pine and spruce pollens was made with aid of 640X magnification. While a separation was made of small and large pine pollens as suggested by Cain (2) and Buell (1) there is still great doubt in the mind of the author if *Pinus banksiana* can, on basis of pollen size, be separated within a safe margin of error from *P. resinosa*. Pollen of this latter species from Michigan, which was available for examination, has many small pollen grains, some rather aborted in appearance, and so small pine pollen grains present at foot-levels where also large grains had appeared may not be those of *P. banksiana*. It is true, of course, that as a rule when pine pollens first appear in a profile they are strikingly small even to casual observation, differing in general contour from the smaller grains appearing with large grains of pine pollens at higher foot levels. This is true for most interior locations as well as in coastal areas of Maine. The graphs in this study are to be interpreted with such doubt in background.

RESULTS

The great waves of succession up to the initiation of the broadleaved climax are very similar for the whole area considered in this study, and variation in vegetation determined by small latitudinal differences become accentuated during the time when the upper half of the peat deposits were being laid down. Succession proceeded from *Picea glauca* to *Pinus banksiana* to Pinus spp., to deciduous forest cover. Geographical location here introduces variation in the association complexes. Along a convex boundary line determined by the cities Whitehall, Farwell and Midland, Tsuga was a dominant of major importance, associated with Fagus and some Quercus. Its movements are, therefore, again linked with the broadleaved genera. Tsuga usually replaced a minor Quercus peak. Pinus controlled on sandy soil, as it does in these poorer soils in the lake forest today. Quercus increases progressively in controlling significance south of the above-mentioned boundary while Tsuga becomes very sparse in

Abies
Picea glauca
P.mar.
Pinus banksiana
Pinus strobus
Tsuga
Quercus
Fagus/Acer
Ulmus
Larix
Betula
Carya/Juglans/Salix/Fraxinus/Tilia/Unknown
Percentage
1
MEELUSEN FARM
2
BERENS BOG
3
DUCK L. BOG
4
REDMAN FARM
5
DOW MARSH
6
CARROLL BOG
7
AUSTIN BOG
8
BLUE LAKE BOG

Abies
Picea glauca
P.mar.
Pinus banksiana
Pinus strobus
Tsuga
Quercus
Fagus
Acer
Ulmus
Larix
Betula
Carya,Juglans,Salix,Frax.,Tilia,Unknown.
9
10
11
12,
13
MUD LAKE BOG
SCHROEDER BOG
SHELBY BOG
POWELL BOG
FARWELL BOG
Percentage

representation and Fagus declines at a number of stations south of Farwell and the Oceana County locations.

To some extent lesser representation of these genera is compensated by increase in abundance of Cary and Juglans, at least in northern Allegan County. When comparing maxima of Quercus we find an interesting behavior within the zone. Representation ranged from 50% to 60% in the southern limits (Allegan County), decreasing to 20 to 40% along highway 46, fluctuating between 20 to 30% along the tension zone and Oceana County line, and dropping to 10 to 20% along the northern border of the zone in Clare County.

Maximum fluctuations of species and genera within the whole zone are evident in Quercus and Pinus (which one would expect in a tension area). The decline of Ulmus, Fagus and Tsuga (in northern locations) during deposition of the upper foot-levels indicates drier climate, which may be the result of warming (reflected in the increase of Qurecus) towards the time when surface layers were being deposited in the majority of bogs studied along the center of the zone. It may also indicate decreased humidity due to warmer climate. It is a little difficult to explain the striking increase in pine in the upper foot-levels in Allegan County stations. Ulmus follows the pattern found in most areas of eastern and central North America, i. e., a maximum abundance near the middle of the pollen profile. The prominent Betula peak at the surface layers in several bogs may reflect secondary succession following lumbering activities of 75 to 100 years ago.

A fluctuating climate is suggested by the behavior of such key genera as Quercus, Pinus, Tsuga and Fagus, but it was never of sufficient magnitude at any time to involve return to Picea once this genus had declined to relic status. Most strongly favored is the assumption of a drier and warming climate (less available moisture may have been due to lessened humidity). This is indicated by decline of Tsuga and Fagus and the tendency of increase for Quercus. Earlier fluctuations between Quercus and Pinus, or broadleaved genera and Pinus, suggest fluctuations in temperature.

DISCUSSION

Comparisons of pollen profiles from lower Michigan, to the north and south of the tension zone area, at once impress one with the fact that the vegetation has experienced more fluctuation and minor

changes along the tension line than to the north and south of it, and that some climatic factors exert a progressively increasing sharp control to bring about marked "tapering off" changes in forest complex within comparatively small latitudinal distances. As Potzger and Wilson (10) have reported for Third Sister Lake at Ann Arbor, a rather long Pinus period there was terminated by Quercus, and Pinus declined gradually to very low representation, while Quercus showed a rather uniform control to the topmost levels in the lake sediments. The same authors (15) reported only slight fluctuation between Pinus and broadleaved genera (associated with Tsuga) for Middle Fish Lake in Montmorency County and Douglas Lake in Cheboygan County. This indicates that climatic factors there are less varying over larger geographical distances than at the tension zone. It is also quite evident that the climatic values must meet with considerable change within the latitudinal distance of about 60 miles in central Lower Michigan, at least in extremes, which exert control on forest distribution. Quercus here ceases to be a controlling element in the crown cover in forests northward, declining to a secondary representation of only 10% in the southern Clare County region. Livingston (8) says for Roscommon and Crawford counties that there the oaks lack the vigorous growth shown in Kent County. Pinus, on the other hand, southward of the upper limits of the tension zone (at least at some time) declined to relic status in a predominantly Quercus crown cover (Meeuwsen and Berens bogs). Along the central section of the belt the two genera (Quercus and Pinus) fluctuate in importance with time (figs. 2, 3). It is also quite evident that this unstable condition has prevailed there for a comparatively long time. Livingston (7, 8) sensed this tension zone and its unique characteristics when he planned the detailed study of the vegetation of Roscommon and Crawford counties in the north and Kent County in the south. He states that Kent County is the northern limit of many typically southern plants, and it is traversed by the southern boundary of the pine forest region. Roscommon and Crawford counties are somewhat north of the Clare County northern limit of the present study. He concludes his excellent observation by saying: "A study of the transition zone between these two areas will be necessary before the working out of the exact relation of the various societies can be attempted." It is, of course, unfortunate that no study similar to those recorded by Livingston was made for one or more of the inter-

vening counties, it would augment greatly the value of the historical picture as it were projected into the present.

The typical lake forest (Tsuga-broadleaved forest and Pinus associations) as we know it today was not well expressed along the tension zone and southward of it (fig. 2). Tsuga here does not appear in the pollen profile with more than 5%, but apparently it was more abundant in its southward range along the lake shores than in interior locations, as shown by the Oceana County bogs and the Dow Marsh station in Midland County. In the interior locations abundant representation of Tsuga arched northward about 20 miles. This definitely points to a humidity control, with greater saturation along the lakes. Of course, Tsuga continues to decline in abundance southward until in Indiana it is never represented above 2%, and this in scattered distribution throughout the pollen profile for a given bog.

Livingston (7, 8) leans heavily on soil factors for his interpretation of the forest type distribution, and it seems as if he overlooks entirely the master control, climate. Sandy soil in Kent County and sandy soil in Roscommon County would hardly respond with identical vegetation because climate would militate against Quercus in the northern counties but not in Kent County. Likewise, sandy areas in northern Indiana, even in inland locations, once were occupied by Pinus and this was replaced by Quercus, apparently after moderating climate had made this possible. As Potzger (9) has shown in pollen profiles, when Pinus was climatically favored in northern Indiana, in southern Michigan, and also in northern Michigan, it was the controlling forest cover on all types of soil. Being limited to sandy soil, as in Kent County, is an incipient relic status. There is every evidence that the forest tension zone is also climatically transitional, where a number of climatic factors, especially extremes, are closely spaced latitudinally, and the forest is merely expressing the biological values of these environmental factors. One is greatly impressed with the many meteorological phenomena which have important dividing lines at or near the Saginaw-Muskegon tension zone, and this must find expression in biological values. Just to mention a few: The line for average depth of 40 inches of frost penetration for January runs a little south of the tension zone (Visher, 12); the tension zone is the divide between average date of beginning of warm weather (daily normals above 50 degrees F) on April 1 to the south

and May 1 to the north (Visher, 11) ; a little south of the tension line is the divide between beginning of summer (daily normals above 68 degrees F) on June 15 to the south and July 1 to the north (Visher, 11) ; a little north of the tension zone is the separating line for end of summer coming August 15 northward and September 1 southward (Visher, 11) ; the average number of summer days is 50 at the tension line and 75 to the south (Visher, 13) ; days with temperatures constantly below 32 degrees F (per normal year) in the shade are 60 at the zone limit and 30 southward (Visher, 13). The zone separates regions of long winters, where soil freezes 3 to 6 feet, and region of milder winter, where soil freezes only 18 to 36 inches (Visher, 13) ; it is the dividing line between dates when 3 inches of snow cover the ground, being Dec. 1 northward and Dec. 15 southward (Visher, 14) ; it is also the dividing line for dates after which snow no longer covers the ground, being April 1 northward and March 1 southward (Visher, 14). Significant other differences in temperature between the southern and northern limits of this arbitrarily established zone are also evident in the long time observations of weather reports (5). The growing season is ten days longer at St. John than at Alma (about 30 miles difference), and 13 days longer than at Harrison (northern limit of zone). The average last killing frost is 5 days later at Alma than at St. John, and 8 days later at Harrison than at St. John. For other significant differences see table I. These differences in temperature phenomena dealing with shorter growing season, but especially with periodic greater extremes in late and early frosts must exert a tremendous control on more sensitive southern broadleaved genera, such as Quercus, thus favoring the pines.

For the southern limits of the zone we have a fine description of the primeval forest by Livingston (7), and for a location somewhat north of the northern limits the same author (8) left an excellent map record of distribution of forest types (Roscommon and Crawford counties).

Livingston (7) presents in a detailed map upland forest associations and their distribution in Kent County, the least common of the several associations he calls the oak-pine-sassafras, and it is found on the poorest soil sites. It appears in small colonies in the southern part of the county and has greatest abundance in the northern part. He summarizes by saying, "In the northern part of the county white pine was almost universally present in the uplands at the time of

settlement." In Roscommon and Crawford counties (8) he found conditions quite different, the southern oak forest was almost wanting, there was lack of "tension," except for the competition between the three species of Pinus. Broadleaved forest was a very negligible factor in the forests of the counties. He sums up by saying, "The prominence of the pines in the region under discussion is an expression of the fact that the flora here is a typical northern one. Only one pine (white) is found in Kent County, and there it grows in poorer soil than it holds here."

As one ponders on all climatic and vegetational phenomena which intermingle at the tension zone, one realizes that it must be a southern limit of many atmospheric disturbances associated with the sweep of "highs" across our continent, and that it is not a "time" factor influencing the progressive northward move of plants. The control is rather due to a climatic "ridge" with sufficient difference in climatic values to the north and south of it to induce a "marking time" forest condition. Small temperature variations in the past have found expressions in decline or advance of coniferous and broadleaved genera, and variation in available moisture initiated advance or retreat for Tsuga and Fagus.

About mid-profile most of the bogs at and north of highway 46 show a Quercus peak, this probably represents a warmer or drier period; these same bogs also show an increase in Quercus at topmost levels, which suggests a warming climate. For years the author has wondered about the marked change in forest cover in Muskegon County where pinelands of a century ago show a marked succession to Quercus. This can hardly be a result of lumbering and fires. In counties a short distance to the north, as well as in northern Wisconsin, such destroyed pine forests of the past are not being replaced by Quercus, but these lands have been lying idle, vegetated at best with aspen or sweet fern, bracken and various species of Vaccinium. According to reports of the American Geophysical Union (1942) there is a general retreat of glaciers in North America, both in our western mountains as well as in Alaska. It is the writer's opinion that change in forest type is the logical thing to expect along a sensitively balanced border area like central Lower Michigan when small changes in climate occur.

SUMMARY AND CONCLUSIONS

1. A pronounced *Picea glauca* period represents the initial forest vegetation over the whole area involved in this study.

2. *Picea mariana* shows greatest abundance in the foot-levels of the lower third of the profile in all of the 12 bogs which are represented with a complete profile.

3. Picea was always associated with low percentages of Pinus, even during early history of the bogs studied.

4. *Picea glauca* was replaced by *Pinus banksiana* in all of the 12 bogs.

5. In some localities *Pinus strobus* (or *P. resinosa*) replaced *P. banksiana* dominance; in others it merely depressed abundance of *P. banksiana*.

6. Broadleaved genera and Tsuga replaced the Pinus period.

7. The tension zone marks the southern limits of important Tsuga representation, never exceeding 5% south of highway 46, but reaching 31% at Powell Bog, 23.5% at Farwell, 27.5 at Dow Marsh, 34 to 58% in Oceana County. There was, thus, a tendency for Tsuga to show greater abundance southward along the lake shores than in the interior.

8. Tsuga usually replaces a Quercus peak, but it shows a tendency to decline during the more recent past.

9. Fagus has greatest abundance northward, with two peaks as characteristic feature.

10. Acer attains greatest abundance in the Oceana County area.

11. Quercus exhibits considerable fluctuation with a tendency (in most bogs) to increase towards topmost levels.

12. Ulmus shows greatest abundance at mid-profile levels, with decline towards surface layers.

13. A high Betula representation in samples from the Austin and Powell bogs may indicate vegetational changes due to lumbering.

14. Carya and Juglans have significant increase in mid-profile with decline at upper third levels in the Meeuwsen and Berens bogs. At all other stations a consistent but sparse representation is characteristic.

15. Tilia is sparsely represented at all locations.

16. There is no indication of decided cooling of climate during recent post-Pleistocene times (no return to Picea anywhere) but drier climate is suggested by decline in the upper foot-levels of Tsuga

and Ulmus and increase in Quercus (increase of Pinus in southern locations).

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TABLE I

Variations and extremes in temperature phenomena in the tension zone area of Lower Michigan.

Stations	Average date of last killing frost	Average date of first killing frost	Average length of growing season in days	Latest date of killing frost	Earliest date of killing frost	Lowest temperature in Fahrenheit J	F	M	A	M
Holland	5-8	10-8	153	5-27	9-17	–17	–24	–8	12	21
St. John	5-7	10-6	152	5-29	9-21	–16	–22	–7	10	25
Muskegon	5-1	10-16	168	5-27	9-16	–21	–30	–11	9	22
Hart	5-17	10-7	143	6-23	9-2	–26	–35	–16	3	18
Alma	5-12	10-1	142	5-29	9-10	–24	–29	–10	6	21
Saginaw	5-1	10-7	159	5-26	9-14	–15	–23	–5	8	24
Midland	5-13	10-1	141	6-6	9-1	–24	–30	–20	5	23
Gladwin						-28	–39	–17	5	21
Harrison	5-15	10-1	139	5-30	9-4	–20	–36	–16	–6	20

FORESTS OF THE PAST ALONG THE COAST OF SOUTHERN MAINE[1]

By John E. Potzger and Ray C. Friesner

Pollen anlysis in North America is gradually adding more and more significant facts on the history of our forests in glaciated North America, showing likenesses and differences in succession in the various geographical locations; throwing into bold relief definite patterns of forest development for a given region or for several regions. Forest types also suggest climatic changes and fluctuations which made such forest types possible. Up to the present our eastern coastal areas received less attention from the pollen analyst than the interior central states. The analysis of peat from New Jersey by Potzger and Otto (7) and the studies by Deevey (3, 4) in Connecticut made at least a beginning in pollen study along the Atlantic coastal areas. They also accentuate the need of further studies along our eastern border to add to the completeness of the picture with respect to forest succession during post-glacial times, especially northward of the New Jersey and Connecticut stations. The present study has this expansion of area as its specific aim.

A series of six bogs, located near tide water of the Maine coast, ranging in geographical distance from Mt. Desert Island on the north to west Rockport on the south, constitutes the field of study (fig. 1). These bogs are no doubt representative records of the history of the forests along coastal areas in southern Maine for all present essentially the same story.

PHYSIOGRAPHIC AND GLACIAL FEATURES OF MAINE

The coast of Maine is frequently very rugged, with peaks rising up to 2,000 feet elevation a short distance inland from tide water.

[1] This is contribution 198 from the Botanical Laboratory of Butler University. The field work was supported by a research grant from the AAAS through the Indiana Academy of Science, for which the author is deeply grateful. We also express our sincere thanks to Esther Whitney Potzger for the lettering on the graphs. We are further appreciative of the co-operation given us by Mr. Gershom Walden and the assistance received from the publication by Trefethen and Bradford (8) to locate the bogs.

Late Wisconsin glaciation determined most of the soil and the topography of the more level areas. Much of the soil, especially on slopes, is shallow and rich in glacial boulders. According to Trefethen and Bradford (8) all of Maine, even Mt. Katahdin, was over-ridden by Late Wisconsin glaciers.

FORESTS OF THE REGION STUDIED

The forests of today are a mixture of coniferous and broadleaved species, bearing the general aspect of the lake forest, to which the forests of Maine are no doubt closely related. *Picea rubra* plays a prominent role, but *Picea glauca* and *P. mariana* as well as *Abies balsamea* are represented abundantly in the forest complex, and they are associated with *Pinus strobus* and *Tsuga canadensis*. These forests are a result of secondary succession after lumbering eliminated the primeval forest. Growth of *Pinus strobus* is very rapid in this part of Maine. Ring counts of a number of stumps which were 32 inches in diameter gave the average age of the trees as 70 years. On the mountains the forest types are frequently sharply separated into various associations, this segregation is apparently determined by microclimatic factors.

THE BOGS OF MAINE

The bogs of coastal Maine differ very much as to origin, configuration and early sedimentation processes from any which have come to the attention of the authors. The extinct as well as extant lakes are strikingly elongated with their long axis inclined from northwest to southeast. Most bogs are of the valley type, some of them covering extensive areas; these latter ones are locally called "heaths." Nearly all bogs have twelve feet or more of bluish-gray rock flour, frequently referred to as "blue clay," as initial deposit. Microscopic examination of this bottom sediment shows that it consists of small, very sharp, quartz crystals. The material is of putty-like consistency, and this makes sampling and cleaning of borer a distinctly difficult problem. The peat rests, sharply separated from it, on the rock flour layer. The maximum fill was 33 feet. Abundant diatom flora is a characteristic of most of the bogs.

Valley-type bogs are difficult to sample because the deepest part of the depression is not easily located, this is however, an essential requirement if a complete pollen profile is to be anticipated. In

most instances several test borings were made to determine the general slope of the basin floor.

Evidently all the coastal bogs were under the influence of wasting glacier front for a long time. This is suggested by the usual deep layer of rock flour, and is also confirmed by the interpretation given by Trefethen and Bradford (8). The surface of the mat is covered with Sphagnum and dense growth of small shrubs, of which *Rhododendron canadensis, Chamaedaphne calyculata, Spiraea salicifolia, Kalmia angustifolia* and *Ledum groenlandicum* are most common and most abundant.

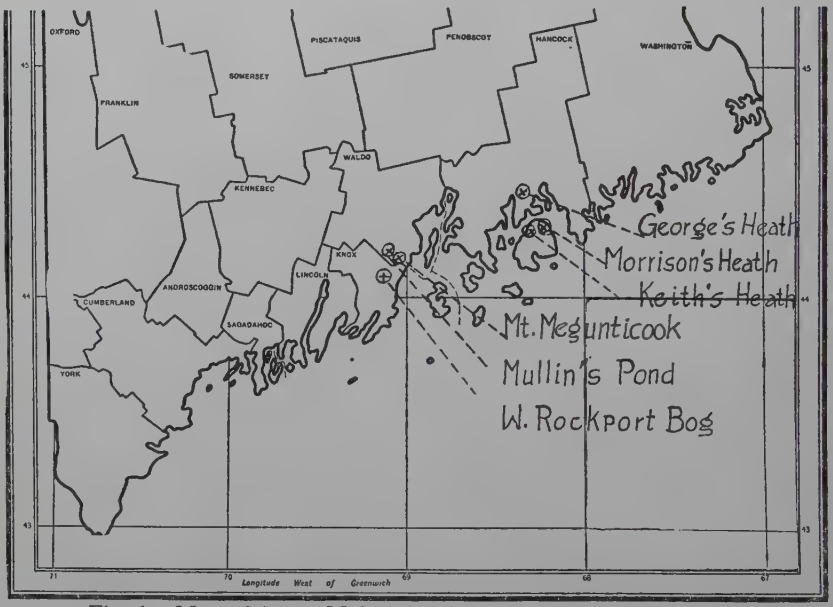


Fig. 1. Map of lower Maine showing location of bogs studied.

Keith's Heath

This bog is located on Mt. Desert Island about two miles west of Morrison's Heath and about two miles south of the Mt. Desert Island Biological Laboratory (fig. 1). The large, oblong area is devoid of all tree vegetation and has a dense cover of Sphagnum and shrubs. A layer of peat totalling 12 feet in depth rests on a 17-foot layer of rock flour.

Morrison's Heath

The extensive heath is also located on Mt. Desert Island, about two miles east of Keith's Heath (fig. 1). The vegetation is composed of a dense shrub association in which scattered tree invaders have made their appearance. The water table is low and so Sphagnum has a depauperate appearance. A dense forest surrounds the heath. The associates here are *Pinus strobus, Picea glauca, P. mariana, Larix laricina,* and *Pinus resinosa* while a dense tangle of Alnus forms a fringing zone.

George's Heath

A sweeping valley, bordered by straggling forest, marks George's Heath. It is located in the Town of Franklin at the junction of highway 200 and the Franklin-Eastbrook town line. Length of the bog extends several miles and its width is at least a half-mile. The boring was made in the approximate center of the trough, about a half-mile from the northwest border. Dense cover of Sphagnum and a prominent low shrub association constituted the vegetation of the mat.

Mt. Megunticook Bog

The bog is located on the northwest slope of Mt. Megunticook, a half-mile south of the trail to Bald Rock mountain, in Knox County (fig. 1). A dense forest, composed primarily of *Picea rubra, Acer rubrum,* with *Pinus strobus* and *Betula papyrifera* showing secondary importance, covered the bog surface, while a luxuriant growth of Sphagnum formed the surface layer. The border forest is an association of *Pinus strobus, Betula papyrifera, Tsuga canadensis, Fagus grandifolia* and some *Quercus borealis* var. *maxima.* Peat totalled about 17 feet and rested on a three-foot layer of rock flour.

Mullins Pond

This long valley-type bog is now almost completely covered by a mat. A small sluggish stream flows through the central area and its waters spill over a rock basin rim before entering the Lake Megunticook lowland. It is located a quarter mile north of the eastern lobe of Lake Megunticook in Waldo county. The bordering forest is an association of *Pinus strobus, Picea glauca, Tsuga canadensis, Fagus grandifolia, Acer rubrum* and *Betula papyrifera.* The boring was made near the center of the trough about 200 feet west

of the eastern rim of the basin. Organic deposits totalled 21.5 feet which rested on a nine-foot layer of rock flour.

West Rockport Bog

Two miles west of West Rockport, and a half mile north of highway 37 is located a long valley-type bog, ranging in width from a quarter to a half mile. The surface is still very wet and is covered by a dense growth of Sphagnum. At some places tall shrubs cover the mat. The deposit of rock flour was deeper here than in any other bog studied, it totalled 24.5 feet. The boring was made in the center of the trough.

METHODS

Sampling was carried out with a Hiller-type borer. The outer layer of the core within the cylinder was cut off with a knife and the sample was thus secured from the central portion of the core. The peat samples were placed into properly labelled 1-oz. bottles. As a rule samples were taken at one-foot intervals, except where sharp differences in appearance of the sediment make more frequent sampling advisable. The laboratory technique followed closely the Geisler (5) method. A 5% aqueous solution of gentian violet served as stain. Coarse peat was strained through cheesecloth to remove large plant fragments. Tabulation was made with aid of 150X magnification, but all small and not-easily-identified pollen grains, as well as all measurements of Pinus and Picea pollens were made under 640X magnification. While all small Pinus pollens were classified as those of *Pinus banksiana,* according to Cain (2) and Buell (1) there is serious doubt in the mind of the author whether this is a reliable means of differentiating when pines with larger pollen grains make their appearance, for pollens of *Pinus resinosa* overlap the size range of *Pinus banksiana.* A count of 200 pollen grains was made for most foot-levels except where pollen representation was sparse. In the rock flour deposits pollen was frequently very sparse. There, 10 slides were used for data in tables 1 to 6. It was concluded that the small representation indicates a very sparse vegetation cover of the mixed forest type which invaded the newly exposed land surfaces.

OBSERVATIONS

No previous series of bogs described by the writer presented so

uniform a forest succession as the pollen profiles from the bogs included in this study (figs. 2 and 3, tables 1-6). Pollen representation was sparse in most lower foot-levels where sediments consisted of rock flour (tables 1-6) but a mixed forest type, including both coniferous and broadleaved genera, even Quercus, is suggested by the pollen representation. Beginning with the layers of organic sediments the dominants in the forest were *Picea glauca* (primarily) *P. mariana* and *Pinus banksiana.* This association persisted while four to six foot-levels were being deposited. This was succeeded in all bogs by a prominent *Pinus strobus* (*P. resinosa*) peak in which Tsuga and Betula played an important role. In general, *Pinus banksiana* had greatest abundance of pollen in lower foot-levels; its initial decline coincided with that of *Picea glauca.* However, unlike that of Picea, it showed no later recovery; also, it did not reach the significant representation of the taller pines. *Picea mariana* lacked the abundance of *P. glauca.* It fluctuated more, but was like it in a secondary peak in upper foot-levels. Tsuga has two prominant peaks, separated by decided decline in abundance. In the more recent past it apparently assumed a very secondary position in the forest complex. Quercus suggests greatest abundance at midprofile levels, but at four stations (fig. 2 and 3) it indicates recovery at surface levels. It was an early component of invading tree groups, as a rule, already at bottommost levels where sediments were composed of rock flour. Betula plays an important (with some fluctuations) role throughout most of the seres in forest succession. While a faint tendency to show two peaks and declines is noticeable, these are, however, not so prominent as in Tsuga. Abies plays a consistently unimportant part in the forest complex. The most striking single feature of all profiles is the pronounced double peak for *Picea glauca*: one at lowermost levels, which is succeeded by a sudden decline which is so characteristic of stations in the Central States, leading to a long period bordering on relic status, and this is followed again by a striking increase in abundance of pollen at one-foot and surface levels (figs. 2 and 3). Of other broadleaved genera besides Quercus and Betula, only Fagus suggests the semblance of an important participation in crown control. Its invasion is associated with decline of *Pinus strobus* and Quercus. This occurs at midprofile. Carya, Juglans, Tilia, Acer and Ulmus occupy positions of secondary importance. The forest, thus, changed from a mixed coniferous type

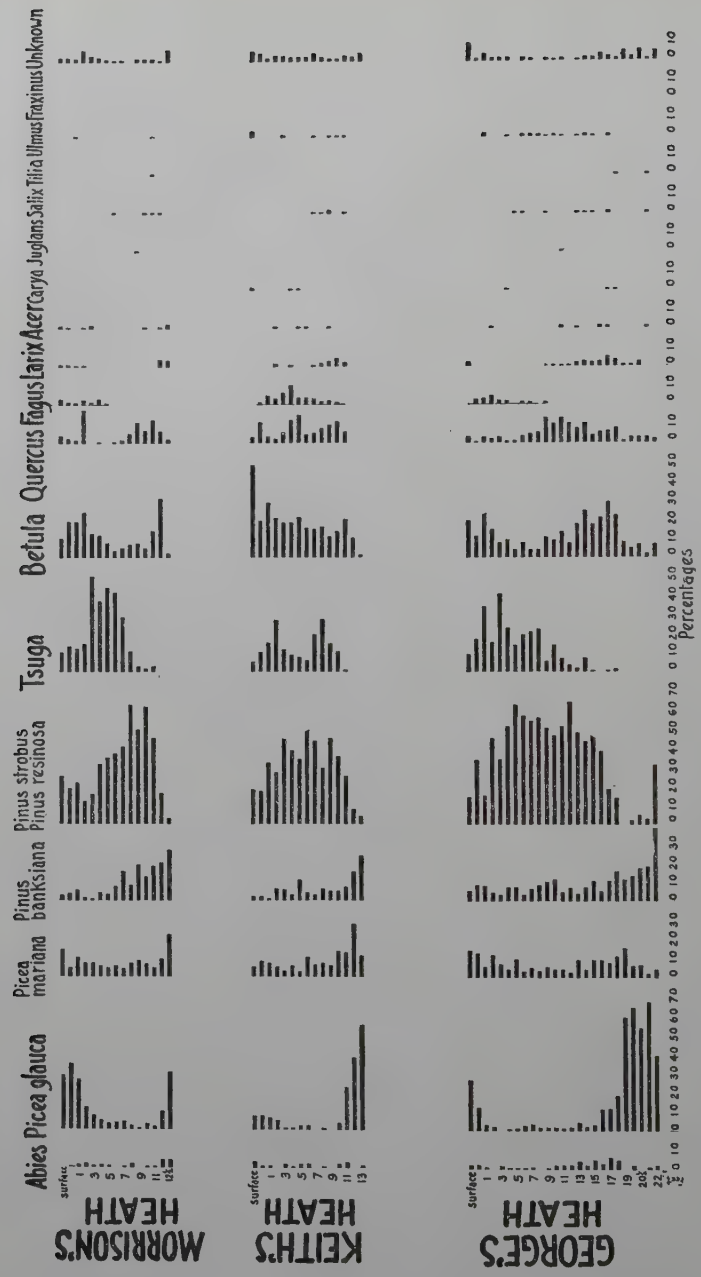


Fig. 2. Profiles from the three northernmost bogs.

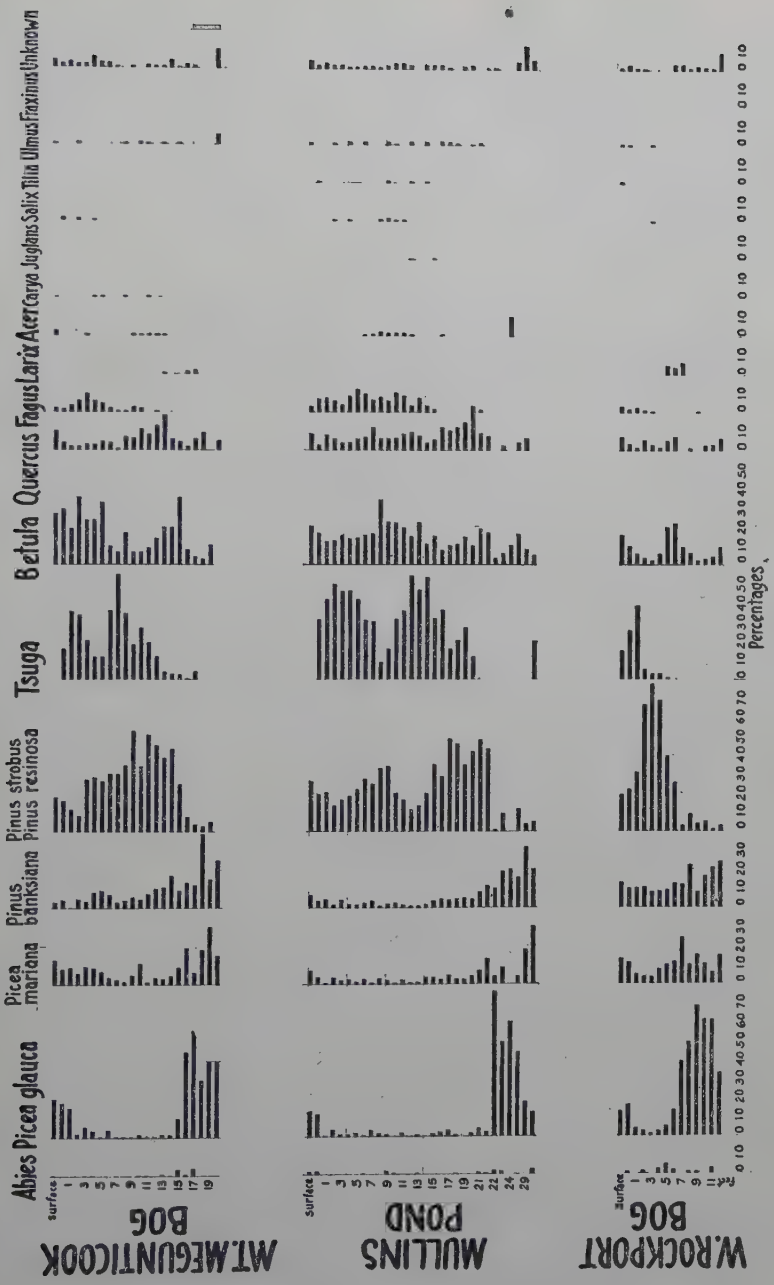


Fig. 3. Profiles from the three southernmost bogs.

to one constituted primarily of tall pines, which in turn yielded to a *Pinus strobus*-Tsuga-Betula association in which Betula was briefly replaced by Quercus. Betula and Tsuga alternate in two peaks of abundance, in which Betula probably represents a drier period. The final succession was to an association of conifers and broadleaved genera, with a decided increase in Picea while the topmost foot-level of peat accumulated.

DISCUSSION

The initial postglacial forest along coastal areas of eastern North America was not the simple *Picea glauca* consociation which one finds universally in the central states. Potzger and Otto (7) refer to a similar condition in northern New Jersey and attribute it to more rapid moderation in climate along coastal areas. The rock flour deposits originated without doubt from melting glaciers, and since pollens of Tsuga, Quercus and Betula are found in these sediments we must conclude that climate in coastal Maine must have moderated more rapidly than in interior locations. During the period of emergence of land surfaces from the ice cover a sparse tree vegetation advanced northward. While glaciers were retreating and rock flour sediments accumulated in the basins of lakes a sparse tree vegetation invaded the ice-free borders. The components were primarily conifers but Betula and Quercus found climatic conditions tolerable to their presence. A unique feature of the succession is that the Picea climax (especially *P. glauca*) followed a mixed association of conifers with sparse representation of broadleaved genera. The broadleaved genera and Tsuga persisted throughout the Picea climax in secondary status but the increase in abundance of pollen indicates a status of codominance in later successional stages. Rapid advance of forests after melting back of glaciers is suggested by the abundance of pollen in the organic deposits which rested without a transition zone on the rock flour deposits.

Behavior of Quercus differed greatly in Maine from that in New Jersey. While in Maine it never assumed a role of dominance, it had its highest sepresentation in midprofile of all bogs, it was associated with *Pinus strobus* during the period of its highest representation, which came before the first peak representation of Tsuga. In New Jersey it suggests a climax condition to succeed Pinus. While Tsuga is much more prominent in Maine than in New Jersey its

abundance distribution is very similar, more or less indicating two periods of more favorable climatic conditions followed by some depressing factor. In Maine this may mean periods of more abundant available moisture alternating with periods of reduced available moisture. Potzger and Otto (7) postulated similarly for New Jersey under similar expressions, and involving, thus a wider geographical area than the lower New England state. Present climatic conditions do not appear favorable to Tsuga. According to Hyland and Steinmetz (6) the range of *Pinus banksiana* is at present found within the northern limits of the area represented by the bogs listed, but only in scattered colonies, indicating that the taller pines reduced its abundance when climate became favorable to their maximum development. Here as in the central states, the Pinus peak is so pronounced and of such long duration (figs. 2 and 3) that one can hardly escape the assumption that Pinus constituted a climatic climax which was replaced when moderating climate favored Tsuga and broadleaved genera.

The pollen profiles of Maine present a striking and consistent alternation between Betula and Tsuga during peak representation (figs. 2 and 3) indicating replacement of Tsuga by Betula, or rather by a Pinus-Betula association during drier climatic cycles. If we assume as correct the postulated xerothermic period during postglacial times we may read it into the alternations of Pinus-Betula and Tsuga dominance. However, we would then be obligated to assume several such periods. It may also be read into the higher representation of Quercus at midprofile positions. The author feels that the pollen analysis records are at present too meager to warrant basing on them the sweeping generalizations whih at times are expanded into regional dimensions from records of a few bogs in any one locality.

Fagus is a more recent invader into the forests of Maine but appears to be a rather discouraging indicator of climatic values. From the acquaintance with it in Indiana, Michigan and Wisconsin one would expect its increase in abundance to reflect a climate marked by greater moisture abundance, but in Maine it develops into greater represntation during a period when the reduced percentage of Tsuga pollen induces one to postulate reduced available moisture. Since moisture is not the sole criterion of controlling force of distribution of genera, but temperature, too, plays a vital part we are at least justified to say that the combined climatic factors were more favor-

able to the invasion and ecesis of Fagus during the period of time during which the upper third of the peat deposits accumulated than during the period preceding, and that conditions which prevailed in the most recent past have not been so favorable to its competitive participation in the forest cover as at a former period.

The striking profile configuration for Picea, especially for *Picea glauca*, at first thought suggests a decided cooling during recent times, however, other explanations must be reckoned with in an attempt to account for the important increase in Picea pollen while the topmost foot-level of peat accumulated. *Picea rubra* is an important constituent of the forests of lower Maine, it is more tolerant than either *P. glauca* or *P. mariana*. Taxonomic characters as well as comparisons of its pollen with that of the companion species of Picea indicate a hybrid status. The size range of *Picea rubra* pollen includes that of both *P. glauca* and *P. mariana*. There is, thus, the possibility that the striking increase in Picea pollen at the closing foot-level marks the entrance of this more tolerant species (hybrid,) into the forest composition of the East. On the other hand, this Picea "peak" at closing foot-levels does not appear in the profiles from northern New Jersey (Potzger and Otto, 7), neither does Deevey (3, 4) find it universally present in Connecticut. There are, of course, other features of the profiles which might be interpreted as indicators of a cooling climate in more recent times. These are: the decrease of Fagus, Tsuga and *Pinus strobus,* the more frequent occurrence of Carya and Juglans in the middle third than in upper third of the profiles. Militating against such interpretation are the increase in Betula and Quercus at upper levels and the present northward distribution of Carya and Juglans. Hyland and Steinmetz (6) include southern Somerset and Franklin counties for *Carya ovata* and southeastern Oxford and western Kennebec for *Juglans cinerea.* If accurate data on rate of peat accumulation were available it might be found that the upper foot-level of peat accumulated since civilized man began his lumbering activity in Maine some 300 years ago. Increase of Picea in that case would, perhaps, mean greater abundance of Picea in response to lessened competition from more tolerant trees.

SUMMARY AND CONCLUSION

1. The study reports on pollen analysis of peat from six bogs located between Mt. Desert Island and W. Rockport, Maine, in which extreme correlation of profile characteristics is an outstanding feature.

2. All bogs record initial sediment composed almost entirely of rock flour which in some instances totalled 20 to 24 feet.

3. Pollen representation is very sparse in the rock-flour layer but that which is present indicates a forest composition consisting of coniferous and broadleaved genera; even Quercus is consistently a component of these invading forests.

4. Forest succession followed a general pattern: Picea-*Pinus banksiana* to *Pinus strobus* (P. resinosa), to *Pinus strobus*-Betula-Quercus, to *Pinus strobus*-Tsuga-Betula, in which Tsuga and Betula alternated in representation, to a mixed coniferous-broadleaved forest complex, consisting of Picea, Pinus, Tsuga, Betula, Quercus, and Fagus.

5. Profiles of Tsuga and Betula show two more or less pronounced alternation "peaks" of abundance, and the opinion is expressed that they indicate alternating cycles of dry and moist periods.

6. Quercus appeared as a component of the invading forest type. While it never assumed great prominence, it shows highest abundance at mid-profile foot-levels and an increase in abundance at the one-foot levels.

7. Abies, Carya, Juglans, Tilia, Acer, Ulmus, Larix and Fraxinus were always sparsely represented.

8. *Picea glauca* had two periods of important representation: one in the initial forest and a second at the one-foot and half-foot levels. It is suggested that this may indicate a cooler period in recent geological time, or it may mark the advent of *Picea rubra,* which shows a number of pollen and anatomical features to suggest its being a hybrid between *Picea glauca* and *P. mariana.* Pollen grains of *Picea rubra* overlap the size-ranges of the other two Piceas.

9. A cooling climate during recent geological time may also be read into the decline of Fagus, Betula and Tsuga in the upper quarter of the profiles. However, the tendency for Quercus to increase in abundance in the topmost levels militates against such a conclusion.

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TABLE I

Morrison's Heath

Numbers of pollen grains counted at any given foot-level.

Foot-level	Surf	½	1	2	3	4	5	6	7	8	9	10	11	12	12½	14½
Total no. grains	150	200	200	200	200	200	200	200	200	200	200	200	200	200	100	11
Abies		1	3	4	2	3	2		1	4		1	1	8	4	
Acer	2	1		1	3							1		1	2	
Betula	15	38	37	47	25	23	15	1	10	14	15	10	28	61	2	
Fagus	4	3	2	5	3	6	1		1							
Fraxinus				1												
Juglans											1					
Larix	2	2	1	1										7	3	
Picea glauca	42	68	52	23	14	9	6	7	8	4	1	6	3	19	30	1
Picea mariana	20	9	20	14	14	11	9	11	8	14	17	13	9	18	22	6
Pinus banksiana	4	7	11	3	2	8	7	15	30	16	37	25	36	39	26	2
Pinus strobus (P. resinosa)	37	37	43	24	31	63	70	74	81	125	99	123	90	32	3	2
Quercus	5	5	3	35		1		1	3	10	22	13	24	12	2	
Salix								1				1	1	1		
Tsuga	16	26	24	29	100	74	88	83	57	21	5	3	5	1		
Ulmus			1										1			
Unknown	3	4	3	12	6	4	2	1	1		3		3	1	6	

TABLE I—(Continued)

Number of pollens of shrubs, herbs and spores tabulated while counting above tree pollens.

Foot-level	Surf	½	1	2	3	4	5	6	7	8	9	10	11	12	12½	14½
Alnus	1	4		2		1			4	2	6	2	7	4	6	
Corylus	1	1							1	1	1	1			1	
Ilex		2						2	4	2				2		
Compositae	3	3	2	5	1	2										
Gramineae	4			1		1	1						1		122	
Nymphaea				1									1			
Fern spores					1		2		11							
Dryopteris									2				1	1		
Lycopodiaceae	8		4	4	22											1
Sphagnum		28		1	40	1	2	12							1	

TABLE II

Keith's Heath

Numbers of pollen grains counted at any given foot-level.

Foot-level	Surf.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	20	24	26
Total no grains	100	100	200	187	200	200	200	200	225	200	200	200	200	100	80	10	5	19
Abies	3	1	2		4	1	4	4		1		4	5		1			
Acer				1			1	1			1							
Betula	48	19	57	40	36	36	41	3	32	32	21	27	40	4	1		1	
Carya	1					1	1											
Fagus		1	9	5	12	20	7	7	6	3	4	2						
Larix				2		1			1	3	5	8	4					
Picea glauca	7	7	12	9	1	2	4	4		1	2	7	44	49	45	1	2	4
Picea mariana	5	8	14	10	6	11	5	20	13	14	12	27	25	21	9	1		3
Pinus banksiana	2	2	2	11	11	6	21	8	7	12	10	10	14	16	18	3	1	6
Pinus strobus (P. resinosa)	18	17	64	51	89	77	68	99	97	59	90	71	50	6	3	1		1
Quercus	3	11	7	3	11	24	29	9	12	15	19	22	11	2		2		1
Salix									1	1	3		1					
Tsuga	5	10	30	50	23	18	15	12	44	55	30	21	2			1		
Ulmus	3				2				1		1	1	1					
Unknown	5	4	3	5	5	4	4	4	9	4	2	2	5	3	3	1	1	4

TABLE II—(Continued)

Number of pollens of shrubs, herbs and spores tabulated while counting above tree pollens.

Foot-level	Surf.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	20	24	26
Alnus	13	1	2	2	3	5	7	3	2	3	2	1						
Corylus	2	2		1				1								1		
Ilex	3	16	2	5		3	1	2	1	1	2	1						
Chenopodiaceae											1							
Compositae	7	2		1	2		5			3						1	2	1
Gramineae	14	2	1	4	1	1	1			1			3	29	44			8
Nymphaea						1	1	4										
Fern spores?				1														
Osmunda		5					1		1									
Dryopteris			2			1	3	1										
Sphagnum		1	3	1	3	1												

TABLE III

George's Heath

Numbers of pollen grains counted at any given foot-level.

Foot-level	S.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	19½	20½	21	22	22½	25	29½	33
Total no. grains	100	200	200	200	200	210	200	200	200	200	200	200	200	200	200	200	200	200	200	200	75	100	125	100	100	12	10	3	7
Abies	2	4		2	3	1	1	1	2		1	4	4	4	8	4	9	6	18	9		2		1	2				1
Acer				1									2		1			3	2					1					
Betula	19	22	41	29	15	19	8	15	8	8	21	18	27	15	35	49	35	42	58	44	6	5	9	2	7				1
Carya						1													1	1									
Fagus		5	6	5	4	3		2			2																		
Juglans													1																
Larix	2									1	1	1	1	1	4	5	4	5	10	7	1	1	3			2			
Picea glauca	26	24	6	4		1	2	5	6	3	3	3	2	3	8	4	6	22	23	36	44	64	67	67	39	7	3	1	3
Picea mariana	14	25	8	23	14	9	19	6	10	7	10	8	8	5	18	8	18	18	15	22	11	6	8	2	4		2		1
Pinus banksiana	5	16	12	8	6	15	14	6	12	16	20	22	9	13	7	14	21	10	21	31	8	13	21	18	38	2	4		
Pinus strobus (P. resinosa)	14	67	27	90	68	107	125	113	108	111	101	93	102	128	96	87	93	76	37	28		2	6	3	3		1	2	
Quercus	3	1	6	4	6	2	2	7	5	7	26	20	26	20	15	20	8	11	12	15	1	3	4	3	2				
Salix							1	1			1					1	1		2					1		1			
Tilia																				1									
Tsuga	9	34	61	31	81	48	28	39	42	45	11	28	14	7	4	3	1		1	3									
Ulmus			3			1		2	2	2	1	2	1		1	2	1		2										
Unknown	9	2	7	3	3	3		3	2		2	1	2		1	3	3	7	4	2	4	2	7	1	5				1

TABLE III—(Continued)

Number of pollens of shrubs, herbs and spores tabulated while counting above tree pollens.

Foot-level	S.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	19½	20½	21	22	22½	25	29½	33
Alnus	4	1	2							1		2	1	1	1	4	2	3	5	13	1	3	1						
Corylus	2		1																	1	1			1			1		
Ilex																													1
Compositae	6		4		2			2					1			1			5	1				1	1			
Gramineae	4		4	1		3		2	1	1	2	1	5	1	1	3	2	3	5	31	22	4	14	7	9	4	1		
Nymphaea										3		1	1			3	1	2											
Typha																		3		3									
Fern spores?													1																
Dryopteris	1							2		3										1									
Osmunda				1		1																							
Sphagnum			10	4	3	10	3	2																					

TABLE IV

Mt. Megunticook Bog

Numbers of pollen grains counted at any given foot-level.

Foot-level	Sur.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Total no. grains	200	150	200	150	200	200	200	200	200	200	200	200	200	200	200	200	200	200	150	33	20	20
Abies	1				1	1						1	1		2	1	7	2	5			
Acer	5				1						1	1	1	1	1							
Betula	53	44	38	53	47	47	65	19	13	33	13	13	17	27	39	40	71	15	5		2	
Carya						1	1			1			1									
Fagus	4	2	7	10	19	12	9	4	1		5	4		1								
Fraxinus								1														
Larix													2	1	1			3	4			
Picea glauca	40	26	31	3	11	7	1	8	1	1	1	3	1	7	3	3	20	90	84	10	8	8
Picea mariana	25	13	18	8	19	17	13	7	5	3	10	22	3	7	6	9	18	38	10	6	6	3
Pinus banksiana	6	6	1	7	7	16	18	14	6	8	11	9	15	20	22	34	18	27	18	13	3	5
Pinus strobus (P. resinosa)	36	23	23	13	45	57	53	61	61	70	106	69	102	21	78	87	50	16	7	1	1	
Quercus	20	6	4	4	6	6	9	8	2	14	13	22	17	26	37	12	9	4	8	3		1
Salix		2		1		1		1														
Tilia											1											
Tsuga		25	72	50	41	24	24	72	110	69	37	54	38	24	8	6	5	1	7			
Ulmus	1			1						1		2	1		1		1					1
Unknown	8	3	6	3	4	11	6	5	1		2		3	2	2	8	1	4	1			2

TABLE IV—(Continued)

Number of pollens of shrubs, herbs and spores tabulated while counting above tree pollens.

Foot-level	Sur.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Alnus	8		2	1	1		6	2		1	3		1	2		3	4	3				
Corylus							1															
Ilex	4	24	24	26	28	9	24	3	2	1		1										
Chenopodiaceae											1											
Compositae	5										1		1			1						
Gramineae	10	2	1	2	1	2		1	1	2			1			1	1		3	3		
Nymphaea													2	1								
Fern spores?	1	9	34		6	5										1						
Osmunda	1	4			10	10	14	1														
Sphagnum				1				1														

TABLE V

Mullin's Pond

Numbers of pollen grains counted at any given foot-level.

Foot-level	S.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	21½	22	23	24	24½	26	27	28	29	30
Total no grains	125	200	200	200	150	200	150	200	200	200	150	200	200	200	200	200	200	200	200	200	200	200	200	200	150	200	24	33	25	8	5	50	40
Abies	2	3						1			2	1			1					1			2	1	3	3							1
Acer						1		1	2	4	2	3	3	1				1	3	1		1		1									
Betula	26	33	24	25	25	27	21	31	33	68	35	40	39	30	44	22	30	15	20	23	29	20	38	34	7	12	2	5	4	1	4		2
Carya										1													1										
Fagus	4	14	15	12	7	17	17	19	13	16	10	17	17	7	14	6	3	2		1			2	1		1	1						
Fraxinus			1		2																												
Juglans														1			1																
Larix			1		1	1					2													4				1			1		
Picea glauca	22	23	1	7	1	2	2	1	6	3	1		3		1		2	4	6	1		3	8	5	115	99	10	9	11	3		9	5
Picea mariana	9	7	1	7	4	5	1	5	1	6	2	2	4	1	2	7	7	5	9	5	5	8	14	26	7	17	1	3	1	2	1	9	12
Pinus banksiana	8	7	9	3	6	3	2	5	7	2	4	4	3	2	2	3	7	9	8	9	10	9	16	22	14	38	5	9	4	2	3	16	8
Pinus strobus (P. resinosa)	32	39	41	27	25	37	32	55	50	66	30	36	33	23	27	39	70	58	97	92	70	84	96	87	1	19		1	3			2	2
Quercus	11	6	16	12	7	8	10	14	24	12	10	12	17	19	15	8	11	24	21	24	29	46	18	15		5	2	3	1		3		
Salix				1		1				2	2	1	1					1	1				2					1					
Tilia		1									1			1		1																	
Tsuga	5	63	84	99	68	93	64	62	61	18	26	57	72	109	94	107	64	73	32	41	54	24	1	2	2	6	1	1		1			8
Ulmus	1			2		4		3			1	1		3		3		4	3	2		1	2										
Unknown	5	4	6	4	3	2	1	3	3	2	2	6	6	4		4	4	4	1		3	4		2	1		2		1		6		2

TABLE V—(Continued)

Number of pollens of shrubs, herbs and spores tabulated while counting above tree pollens.

Foot-level	S.	½	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	21½	22	23	24	24½	26	27	28	29	30
Alnus	4	2	4	4	3	2		6	12	1	2	2	4	2	2		4	6	8	3	2	6	5	4	2	1		2	1			
Corylus	1											1					1							1								1
Ilex											1						1																
Chenopodiaceae				1																										1		
Compositae	5	1						2	1					1	1			1						2						1		2
Gramineae	26	14	14	8	12	9	12	9	20	21	18	7	6	7	5	4	1	2	2	1	5		1	2		2		2		1	1		2
Nymphaea		1	2		2	1		1	1	1	1		1		2					1			1									
Dryopteris			6	3	4	3				1		1	1			2			2					1								
Osmunda																						1											

TABLE VI

West Rockport

Numbers of pollen grains counted at any given foot-level.

Foot-level	Surf.	½	1	2	3	4	5	6	7	8	9	10	11	12	14½	15½	18	20	21	28	29
Total no grains	100	200	200	200	200	200	200	200	32	100	150	200	100	33	2	4	9	5	3	3	1
Abies		2		3		5	10	4		1	1		3								
Acer						1	2														
Betula	16	19	11	7	4	11	39	44	3	6	3	6	4	3				1			1
Fagus	3	3	5	2	1						1										
Juglans								1													
Larix			1		1		9	7	2												
Picea glauca	13	31	8	5	2	5	10	26	13	40	102	122	61	11		2	1	1	1		
Picea mariana	13	22	10	7	7	15	20	21	8	10	22	21	6	5	1		1	1			
Pinus banksiana	13	20	20	21	16	17	18	25	4	22	12	33	21	8	1	2	6	1	2		
Pinus strobus (P. resinosa)	19	44	62	133	155	138	79	51	1	9	6	10	1	1				1		1	
Quercus	7	7	3	11	6	3	10	14		1		5	3	2							
Salix					1								1								
Tilia	1																				
Tsuga	16	51	77	10	6	5	1														
Ulmus	1	1			1			1													
Unknown	1	5	2	1			2	6	1	1	3	3	1	3			1			2	

TABLE VI—(Continued)

Number of pollens of shrubs, herbs and spores tabulated while counting above tree pollens.

Foot-level	Surf.	½	1	2	3	4	5	6	7	8	9	10	11	12	14½	15½	18	20	21	28	29
Alnus	5	3		1	1			4		3			1								
Corylus	2							2													
Ilex		2	1																		
Compositae						1											2	1		1	
Gramineae	2	2		1	2	5	5	4	7	5	11	2	2								
Nymphaea							2														
Dryopteris	4		4																		
Fern spores?	1																				
Sphagnum		6																			

ANTIBIOTIC BEHAVIOR PATTERNS OF LIVING CULTURES OF PSEUDOMONAS[1]

By Robert L. Kent

Antibiotic properties of the genus Pseudomonas have long been known, but a review of the literature indicates that *Pseudomonas aeruginosa* has received more attention than any of the other species. This work began as an attempt to obtain other species of Pseudomonas which possess antibiotic properties. This objective was accomplished in the demonstration of antibiotic activity in two cultures of Pseudomonas kept as stock cultures by the Botany Department of Butler Unversity. The first was culture 34 which resembles *Pseudomonas incognito* in behavior, and the second, culture 51 which in many ways is similar to *Pseudomonas myxogenes.*

The second phase of the work was to determine the relationship of pigment production to degree of antibiotic activity and age of the culture. An outcome of these two projects has been a method whereby living cultures of Pseudomonas may be checked periodically with a minimum effect upon the culture.

This paper plots the behavior patterns for the cultures *Pseudomonas culture 34, Pseudomonas culture 51* and *Pseudomonas aeruginosa* over a period of ten weeks.

HISTORICAL

Work on antibiotic substances to inhibit bacterial action dates back to 1878 when Pasteur (10) demonstrated antagonistic action of bacteria against the anthrax organism.

Antibiotic properties of *Pseudomonas aeruginosa* have been known by the medical profession for more than fifty years. Its method of administration, inoculating the patient with a living culture, was sufficiently hazardous to prevent its wide acceptance and its period of use was short-lived. Crude extracts of pyocyanase were used in open wounds for a longer period of time, but variance of preparation of the material and difficulties of keeping it active for any period of time prevented its wide acceptance (10).

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[1] A portion of a thesis submitted in partial fulfillment of the requirements for the master of Science degree in The Division of Graduate Instruction.

The first antibiotic substance recognized as such was the alcohol-, ether- and chloroform-soluble pyocyanase (10). Emmerich and Loew (3), by killing old broth cultures of *"Bacillus pyocyanase"* with chloroform, filtering and concentrating in vacuo, isolated a bacteriolytic enzyme which they called pyocyanase.

Pseudomonas aeruginosa was the first organism found to produce two separate antibiotic agents, the pigment pyocyanine and the colorless pyocyanase. Pyocyanine is considered to be a chloroform-soluble blue pigment, thermostable, filterable and active mainly against gram-positive bacteria. It has a low toxicity when used in vitro.

Formerly pyocyanase was regarded as an enzyme belonging to the class of nucleases (10). The present concept is that pyocyanase is a crude, ether- and alcohol-soluble, thermostable, fatty material composed of a mixture of substances (8) with its activity due largely to unsaturated fatty acids (10).

There have been many compounds isolated from *Pseudomonas aeruginosa* acclaimed as possessing the antibiotic property or as being some of the many compounds responsible for it. Pyocyanine, hemipyocyanine, pyocyanine hydrochlorid, pyocyanine methosulfate (monomethyl sulfuric acid salt of pyocyanine), phenozonium compound and pyocyanine perchlorate (8), pyocyanase, Pyo I, Pyo II, Pyo III, and Pyo IV, (4) are a fair representation of these compounds.

It is generally accepted that the older cultures possess the greater powers of inhibition with the pigment pyocyanine responsible for activity in young cultures, while pyocyanase accounts for it in older ones. Young (11) reports that cultures of *Pseudomonas aeruginosa* not producing pigment did not produce antibiotic activity even when the growth was heavy. In contrast to this condition, Hayes (4) reports non-pigmented cultures are responsible for antibiotic activity. Lapierre (6) reported pigment manifested itself only when favorable conditions existed independently of the species, while Jordan (5) states that it is of no physiological significance that under certain conditions one of the metabolic products happens to be fluorescent. Since it is generally accepted that older cultures possess the greater degree of antibiotic activity, it has been shown that a desirable period of incubation is five weeks with three weeks showing less activity and seven weeks not materially increasing the activity (4). It is also accepted that a culture may lose part or all of its antibiotic activity. This long period of incubation and uncertainty of results

may be an important factor responsible for the lack of commercial manufacture of such a product.

PROCEDURE

Testing discs were punched from Reeve Angel Filter Paper 245 with an ordinary hand punch, making each disc six millimeters in diameter. Discs were not punched from the outer border area of the filter paper because of its uneven texture near the margin. Approximately sixty of these discs were placed in a clean, dry petri dish and autoclaved at fifteen pounds steam pressure for thirty minutes. The discs were not used the same day as sterilized and never more than three days after sterilization. Excess culture material was removed from the test discs by touching to sterile filter paper.

Four culture media were used in this work. Flasks of beef extract-peptone broth for growing the Pseudomonas cultures were prepared from a formula similar to that used by Hayes (4). One-percent Bacto-peptone broth was used for transplanting all cultures; standard nutrient agar was used for plating out *Staphylococcus aureus* and testing the Pseudomonas organisms, and stab cultures on nutrient gelatin were also used.

Four different bacterial cultures were used in this work: *Pseudomonas aeruginosa, Pseudomonas culture 34, Pseudomonas culture 51,* and *Staphylococcus aureus* upon which the Pseudomonas cultures were tested. General descriptions and growth characteristics of the Pseudomonas organisms are listed below.

PSEUDOMONAS AERUGINOSA

1. Short rods 0.5-0.6 μ by 1.0-1.2 μ occurring singly and in pairs.
2. Motile by polar flagella.
3. Negative to Gram stain and acid fast stain.
4. So spores formed.
5. Gelatin stab shows rapid surface liquifaction, heavy pellicle. With increased liquifaction fluid was yellowish, sediment heavy.
6. Agar slant culture thin, flat, glistening; green pigment diffused through medium.
7. Agar plate culture large, thin, glistening; green pigment diffused through medium.

PSEUDOMONAS CULTURE 34

1. Short rods 0.3-0.5 μ by 1.0-1.4 μ occurring singly in pairs and short chains.
2. Motile by polar flagella.
3. Negative to Gram stain and acid fast stain.

4. No spores formed.

5. Gelatin stab not liquified, white surface pellicle, growth heavy along stab line, slight pink color.

6. Agar slant culture thin, moist, glistening, translucent; becoming effuse, yellowish; no green pigment.

7. Agar plate culture small irregular colonies becoming very effuse; no pigment.

PSEUDOMONAS CULTURE 51

1. Short rods 0.5 μ wide by 1.0 μ long occurring singly and in pairs.

2. Motile by 5-7 polar flagella.

3. Negative to Gram stain and acid fast stain.

4. No spores formed.

5. Gelatin completely liquified in seven days; turbidity and sediment; light green pigment through liquified gelatin, blue-green at surface.

6. Agar slant culture beaded, raised, glistening; brilliant blue-green pigment diffused through medium.

7. Agar plate culture subsurface colonies circular, white becoming green in three or four days; surface colonies circular, but rapidly spreading green pigment in colonies and diffused through medium.

These organisms were planted in three separate series determined by the three bulk lots of culture media prepared. Flasks 31, 32, and 33 were from the first lot of media and were inoculated with *Pseudomonas culture 34,* while flasks 41, 42, and 43 were from the second lot of media and were inoculated with the same *Pseudomonas culture 34.* Flasks 51 and 52 were from the second lot of media but inoculated with *Pseudomonas culture 51.* Flasks 71, 72, 73 were of the third lot of media planted with *Pseudomonas aeruginosa,* while *Pseudomonas culture 51* was planted in flasks 81, 82 and 83.

All transplants of Pseudomonas were taken from the stock culture to a 1% peptone broth, incubated for 24 hours at 37.5°C. and one ml. pipetted into each of the test flasks. These in turn were incubated at 37.5°C. for the entire ten weeks period. After the routine of putting the cultures on test each week, sterile 10% glycerine was added to each flask to replace moisture lost during the preceding week. The amount added differed from 8 to 11 ml. and was determined by a mark on the side of the flask from a glass marking pencil. Two to three times per week the flasks were rotated to break up the pellicle which formed after the weekly additions of 10% glycerine.

All transplants of *Staphylococcus aureus* were from the stock culture into 1% peptone broth, incubated 18 to 24 hours at 37.5°C. and inoculated into test tubes of melted agar with a wire inoculating

loop. After inoculation, the melted agar was immediately poured into a sterile petri dish which was then rotated until the agar and bacterial suspension were thoroughly mixed and the agar showed evidence of solidifying. Test plates were considered ready for use any time after the agar had solidified, but at no time was there a lapse of more than one-half hour between pouring of the inoculated agar and the planting of the testing discs. Prior to testing, each flask of Pseudomonas was rotated vigorously at least three times for periods of not less than 30 seconds and with a lapse of time between rotations of not less than 3 minutes. Following the third rotation, the presence or absence of green pigment was recorded. Standard for such recording was kept as simple as possible: 000, absence of green; 001, trace of green; 00X, light green; 0XX, green; XXX, dark green.

Immediately following the recording of pigment, while the viscous mass within each flask was still homogeneous, the cultures were tested for antibiotic production. Testing for antibiotic activity consisted in immersing the 6-mm. discs into the culture of Pseudomonas by means of clean sterile forceps, then removing the excess material from each disc by first touching it lightly to the inside of the flask followed by touching to sterile filter paper, and spacing them evenly, three to a petri dish, upon the solidified surface of the Staphylococcus inoculated agar. Test plates were at room temperature during this time with incubation at 37.5°C. following immediately after application of the testing discs.

Readings in millimeters of the diameter of the clear area surrounding the 6-mm. discs were the basis for determining antibiotic activity (plate I). Best readings were obtained with between 12 and 16 hours of incubation. Longer periods of incubation did not increase the diameter of the area of inhibition although it did increase the growth ring of Pseudomonas surrounding each disc. The resulting additional surface growth seldom exceeded the limits of the clear area and did not increase its diameter, yet it made reading more difficult and consequently less accurate. Figures 2 and 4 had been incubated an additional 24 hours after readings were made.

If conditions prevented reading the test plates within the 12-16-hour period, they were placed in a refrigerator and read as soon as possible after that time. Figure 7 is a test plate held under refrigeration for ten days with no change in either the growth ring or area of inhibition.

RESULTS

Results are tabulated in tables I-III and shown on plate I. Clear areas on the agar surrounding the inoculating discs represent the inhibition zones in which antibiotic substances prevented growth of Staphylococcus colonies. From table I it will be seen that the area of inhibition in all flasks of Pseudomonas Culture 34 appeared when the cultures were two weeks old. At no time during the ten-week period was the area equal to that shown when the cultures were two weeks old. Subsequent weeks showed progressive decrease. The first pigment appeared when the cultures were two weeks old coincident with the first antibiotic activity. Subsequent weeks showed pigment varying from light to dark green with the majority of the flasks medium or dark green.

Results from Pseudomonas Culture 51 are shown in table II and figures 1-6. First antibiotic activity appeared (flasks 81-83) after 96 hours. Flask 51 showed first antibiotic activity after one week but flask 52 showed it for the first time only after two weeks. Correlation between initiation of antibiotic activity and pigment production was not so sharply defined as in Pseudomonas Culture 34. Intensity of antibiotic activity as judged from the average diameter of inhibition rings shows a progressive increase from the time of its initiation until cultures are 8 weeks old when an average diameter of 29.3 mm is reached after which there is a progressive decline.

Results obtained from *Pseudomonas aeruginosa* are shown in table III and figures 7-9. Antibiotic activity appeared in all flasks of this species earlier than in the other two cultures. It will be noted from comparison of data in the table that the activity appeared within 24 hours and continued without great fluctuation throughout the 10-week period of study. Highest averages in diameter of inhibition occurred during the 7th and 9th weeks with averages of 28.5 mm and 29 mm respectively. Pigment production was not evident until the cultures were 2 weeks old and its maximum occurred when they were 3 weeks old. There is no apparent correlation between intensity of pigment and degree of antibiotic activity.

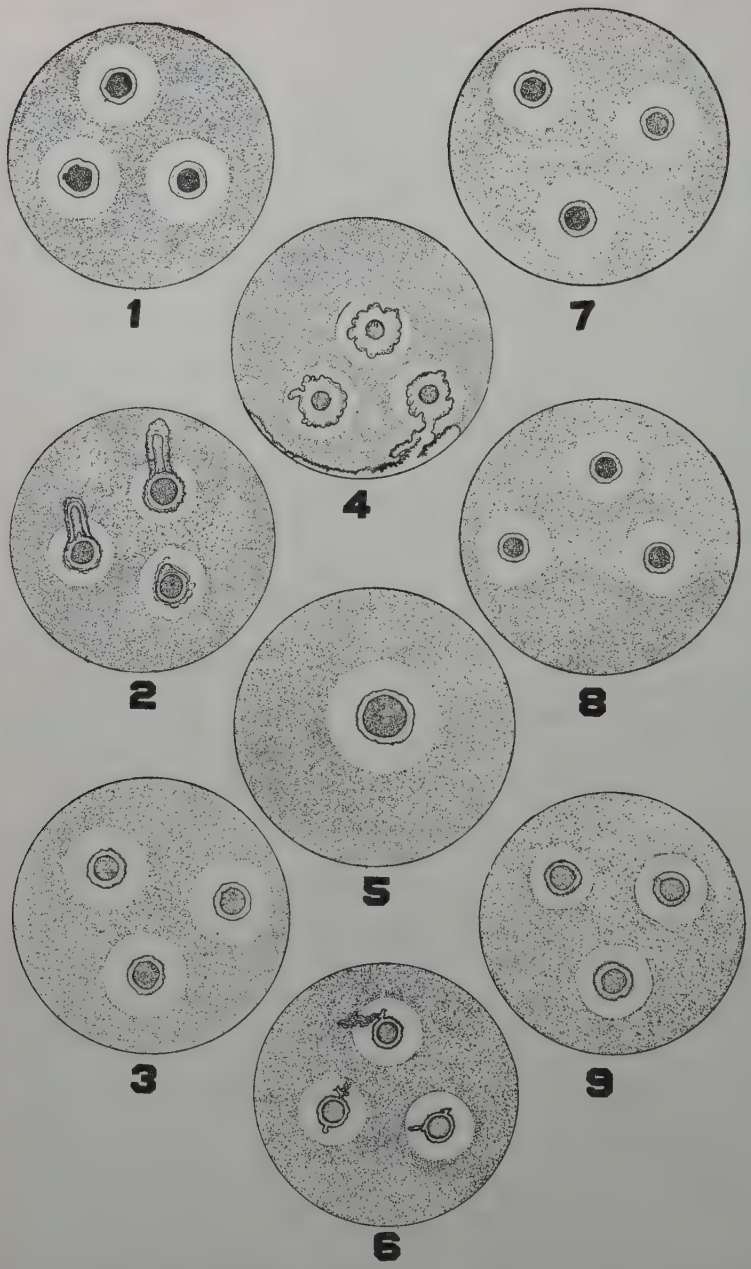


Fig. 1. Pseudomonas culture 51. Flask 51, 9th week. Fig. 2. Pseudomonas culture 51. Flask 81, 6th week. Fig. 3. Pseudomonas culture 51. Flask 81, 8th week. Fig. 4. Pseudomonas culture 51. Flask 82, 4th week. Fig. 5. Pseudomonas culture 51. Flask 82, 8th week. Fig. 6. Pseudomonas culture 51. Flask 83, 6th week. Fig. 7. *Pseudomonas aeruginosa.* Flask 71, 8th week. Fig. 8. *Pseudomonas aeruginosa.* Flask 72, 10th week. Fig. 9. *Pseudomonas aeruginosa.* Flask 73, 8th week.

TABLE I

Pigment Production, Growth and Inhibition data:

Pseudomonas Culture 34

	Flask	Pigment in Flask	Diameter of Growth in mm.	Diameter of Area of Inhibition in mm.
24 hours	31	000[1]	17, 16, 16	0, 0, 0
	32	000	13, 14, 14	0, 0, 0
	33	000	13, 13, 13	0, 0, 0
	41	000	13, 13, 14	0, 0, 0
	42	000	15, 13, 13	0, 0, 0
	43	000	14, 14, 13	0, 0, 0
96 hours	31	000	15, 15, 15	0, 0, 0
	32	000	15, 15, 15	0, 0, 0
	33	000	14, 14, 14	0, 0, 0
	41	000	15, 15, 16	0, 0, 0
	42	000	14, 14, 15	0, 0, 0
	43	000	16, 16, 15	0, 0, 0
1 week	31	000	19, 19, 19	0, 0, 0
	32	000	17, 17, 17	0, 0, 0
	33	000	16, 19, 16	0, 0, 0
	41	000	17, 17, 17	0, 0, 0
	42	000	20, 19, 20	0, 0, 0
	43	000	17, 16, 16	0, 0, 0
2 weeks	31	00x	11, 11, 11	20, 20, 18
	32	00x	12, 12, 11	22, 24, 22
	33	00x	11, 12, 11	19, 20, 20
	41	001	12, 12, 12	17, 19, 19
	42	001	11, 11, 11	18, 18, 19
	43	001	12, 12, 11	21, 21, 20
3 weeks	31	xxx	13, 11, 12	16, 17, 18
	32	0xx	12, 13, 12	17, 19, 16
	33	0xx	12, 11, 11	18, 17, 18
	41	001	11, 12, 11	17, 19, 18
	42	001	12, 11, 12	17, 20, 17
	43	001	12, 11, 10	19, 21, 19
4 weeks	31	xxx	13, 13, 14	17, 17, 16
	32	0xx	12, 12, 13	17, 18, 18
	33	xxx	10, 11, 11	17, 16, 19
	41	xxx	13, 13, 14	16, 17, 17
	42	xxx	12, 13, 12	15, 15, 15
	43	xxx	14, 11, 14	17, 14, 17

TABLE I—(Continued)

	Flask	Pigment in Flask	Diameter of Growth in mm.	Diameter of Area of Inhibition in mm.
5 weeks	31	0xx	12, 10, 12	16, 15, 20
	32	0xx	12, 11, 12	14, 15, 15
	33	xxx	11, 10, 10	19, 15, 16
	41	0xx	11, 11, 11	13, 13, 14
	42	0xx	11, 11, 10	13, 14, 13
	43	0xx	10, 11, 10	12, 14, 13
6 weeks	31	xxx	11, 11, 11	12, 12, 12
	32	0xx	12, 11, 11	15, 14, 13
	33	xxx	11, 11, 11	14, 13, 13
	41	0xx	10, 11, 10	18, 17, 18
	42	00x	13, 13, 12	16, 15, 15
	43	00x	13, 11, 11	21, 21, 20
7 weeks	31	xxx	11, 11, 11	14, 14, 14
	32	xxx	13, 11, 11	17, 16, 16
	33	xxx	10, 10, 10	15, 16, 16
	41	00x	10, 11, 14	14, 14, 16
	42	00x	11, 11, 13	15, 15, 15
	43	00x	10, 10, 11	14, 15, 15
8 weeks	31	0xx	11, 14, 11	13, 17, 14
	32	0xx	13, 12, 10	16, 17, 15
	33	0xx	11, 11, 10	14, 15, 13
	41	0xx	11, 11, 9	14, 14, 15
	42	00x	11, 10, 11	13, 16, 13
	43	0xx	10, 10, 10	14, 13, 13
9 weeks	31	0xx	11, 12, 12	14, 13, 14
	32	0xx	11, 12, 11	13, 14, 13
	33	0xx	11, 11, 10	13, 13, 13
	41	xxx	11, 10, 11	19, 20, 19
	42	0xx	11, 11, 11	17, 18, 17
	43	xxx	13, 13, 13	0, 0, 0
10 weeks	31	0xx	11, 12, 11	14, 15, 14
	32	0xx	12, 11, 11	19, 18, 18
	33	0xx	11, 12, 12	17, 17, 17
	41	00x	10, 10, 10	13, 14, 14
	42	00x	10, 10, 11	12, 13, 14
	43	00x	10, 11, 11	0, 0, 0

[1] 000, absence of pigment; 001, trace; 00x, light green; 0xx, medium green; xxx, dark green.

TABLE II

Pigment Production, Growth and Inhibition data:

Pseudomonas Culture 51

	Flask	Pigment in Flask	Diameter of Growth in mm.	Diameter of Area of Inhibition in mm.
24 hours	51	000[1]	15, 16, 16	0, 0, 0
	52	000	16, 14, 14	0, 0, 0
	81	000	15, 14, 14	0, 0, 0
	82	000	14, 14, 14	0, 0, 0
	83	000	16, 17, 16	0, 0, 0
96 hours	51	00x	15, 16, 16	0, 0, 0
	52	00x	15, 13, 14	0, 0, 0
	81	000	13, 10, 13	17, 17, 17
	82	000	14, 14, 14	18, 17, 17
	83	000	15, 15, 15	18, 18, 19
1 week	51	0x1	12, 13, 12	16, 17, 17
	52	0x1	14, 14, 12	0, 0, 0
	81	001	22, 23, 22	26, 26, 26
	82	001	20, 19, 19	22, 22, 21
	83	001	21, 21, 23	23, 24, 24
2 weeks	51	00x	15, 15, 13	23, 23, 22
	52	00x	9, 9, 9	17, 18, 18
	81	001	11, 11, 11	23, 23, 23
	82	000	12, 12, 11	30, 28, 28
	83	001	10, 11, 11	23, 25, 23
3 weeks	51	0xx	21, 24, 22	25, 27, 25
	52	0xx	10, 10, 12	15, 16, 16
	81	001	10, 10, 11	24, 25, 26
	82	0xx	10, 12, 10	31, 31, 30
	83	0xx	10, 10, 10	25, 26, 25
4 weeks	51	00x	11, 11, 10	23, 24, 23
	52	00x	10, 10, 11	20, 19, 19
	81	001	11, 11, 11	22, 21, 24
	82	0xx	16, 17, 17	31, 30, 30
	83	00x	11, 12, 13	26, 27, 27
5 weeks	51	00x	11, 11, 10	24, 22, 22
	52	00x	11, 11, 11	28, 26, 28
	81	001	13, 13, 13	25, 25, 24
	82	0xx	16, 15, 16	30, 30, 30
	83	0xx	16, 15, 16	30, 30, 30

TABLE II—(Continued)

	Flask	Pigment in Flask	Diameter of Growth in mm.	Diameter of Area of Inhibition in mm.
6 weeks	51	00x	15, 12, 10	27, 27, 27
	52	00x	11, 10, 10	23, 23, 29
	81	001	11, 11, 11	26, 26, 27
	82	0xx	12, 13, 12	32, 30, 30
	83	0xx	11, 11, 10	28, 28, 29
7 weeks	51	001	12, 12, 12	29, 29, 29
	52	001	11, 11, 11	25, 22, 24
	81	000	15, 16, 17	26, 26, 26
	82	xxx	12, 12, 12	30, 32, 32
	83	0xx	14, 15, 15	30, 30, 32
8 weeks	51	000	11, 11, 11	32, 34, 34
	52	000	10, 11, 11	34, 34, 34
	81	000	10, 11, 11	28, 27, 28
	82	xxx	10, 10, 10	27, 28, 28
	83	xx1	12, 10, 11	25, 24, 24
9 weeks	51	000	10, 10, 11	30, 29, 29
	52	000	11, 9, 9	23, 24, 24
	81	000	11, 10, 11	26, 26, 25
	82	xxx	10, 10, 11	28, 28, 27
	83	xx1	11, 11, 11	24, 25, 24
10 weeks	51	000	10, 10, 10	26, 25, 25
	52	000	10, 10, 9	23, 22, 22
	81	000	10, 10, 10	20, 21, 21
	82	xxx	11, 11, 11	28, 29, 28
	83	xx1	11, 11, 11	25, 27, 26

[1] 000, absence of pigment; 001, trace; 00x, light green; 0xx, medium green; xxx, dark green.

TABLE III

Pigment Production, Growth and Inhibition data:

Pseudomonas aeruginosa

	Flask	Pigment in Flask	Diameter of Growth in mm.	Diameter of Area of Inhibition in mm.
24 hours	71	000[1]	17, 18, 18	24, 25, 24
	72	000	21, 19, 20	26, 24, 25
	73	000	18, 20, 20	26, 26, 26
96 hours	71	000	16, 15, 15	19, 19, 18
	72	000	16, 16, 17	20, 20, 20
	73	000	17, 15, 17	19, 18, 20
1 week	71	000	20, 19, 14	27, 26, 23
	72	000	19, 17, 18	27, 25, 27
	73	000	17, 16, 17	24, 23, 24
2 weeks	71	001	13, 14, 13	26, 25, 25
	72	001	16, 15, 15	25, 26, 26
	73	001	11, 10, 11	24, 24, 24
3 weeks	71	xxx	12, 12, 12	27, 27, 27
	72	xxx	12, 13, 12	26, 26, 27
	73	xxx	15, 14, 13	32, 35, 32
4 weeks	71	0xx	12, 12, 12	25, 24, 25
	72	00x	12, 12, 11	24, 23, 24
	73	00x	10, 12, 11	24, 23, 24
5 weeks	71	00x	12, 12, 12	32, 31, 31
	72	00x	12, 11, 11	25, 22, 23
	73	00x	11, 11, 11	24, 25, 25
6 weeks	71	00x	12, 12, 12	23, 23, 23
	72	00x	10, 10, 10	30, 28, 30
	73	00x	12, 12, 11	26, 26, 26
7 weeks	71	000	11, 11, 11	30, 31, 29
	72	000	14, 13, 13	32, 32, 31
	73	000	11, 10, 11	29, 30, 31
8 weeks	71	001	11, 11, 11	27, 29, 29
	72	001	12, 12, 12	29, 28, 28
	73	000	11, 10, 10	25, 24, 22

[1] 000, absence of pigment; 001, trace; 00x, light green; 0xx, medium green; xxx, dark green.

TABLE III—(Continued)

	Flask	Pigment in Flask	Diameter of Growth in mm.	Diameter of Area of Inhibition in mm.
9 weeks	71	001	11, 11, 12	30, 30, 26
	72	001	12, 11, 12	28, 29, 27
	73	000	12, 12, 11	30, 30, 30
10 weeks	71	001	12, 12, 12	26, 26, 26
	72	001	10, 10, 10	25, 24, 24
	73	00x	11, 11, 11	29, 29, 29

DISCUSSION

The two facts most apparent in *Pseudomonas culture 34* (table I) are that the first antibiotic activity and the first show of pigment came with the second week while the constant decline of activity for the remainder of the ten weeks period showed no response from either the fourth or the ninth week peaks in pigmentation.

Pseudomonas aeruginosa and *Pseudomonas culture 51* showed no relationship between time of initiation of antibiotic activity and time of first appearance of pigment; nor was there any general relation between degree of inhibition and intensity of pigment. This leaves the matter of any correlation between the two activities as doubtful.

Relative merits of the three cultures from the standpoint of antibiotic activity show that *Pseudomonas aeruginosa* had the highest average for the ten weeks period. Flask 73 (table III) had the highest peak for flasks of that series with an area 33 mm in diameter at the third week. The average for *Pseudomonas culture 51* exceeded the average of *Pseudomonas aeruginosa* for the fifth, sixth and eighth weeks with flask 51 (table III) showing a peak of 33 mm and flask 52 showing the highest for all flasks, (34 mm) both at the eighth week. With the exception of the second week, there was no time when *Pseudomonas Culture 34* ever approached the degree of antibiotic activity exhibited by the other two cultures.

The method of testing for antibiotic properties of living cultures is limited by the same factors which govern any other paper disc, agar plate method: first, the lack of uniformity of volume of antibiotic material absorbed per disc; second, that a uniform depth of agar is difficult to maintain; third, that seeding of Staphylococcus may not be uniform; and fourth, the question of irregular diffusion

of the antibiotic material through the nutrient agar. Since these four factors are present in any method of this type, all results are relative and comparable only to the extent that these factors can be controlled.

Greater uniformity in volume of material for each disc might have been attained by sterilization with hot air or by placing the petri dish containing the steam sterilized discs in a drying oven for a definite period of time, however, the method of steam sterilization with partial drying and followed by the period of one to three days before using the discs was used throughout the period of work.

The discs were immersed into the viscous mass, which insured saturation, and then touched lightly to the inside of the flask and followed immediately by touching the saturated disc to a larger sterile disc of filter paper. This probably resulted in fairly standardizing the volumes of the material being carried by each disc.

A more uniform depth of agar was obtained by using 10 ml. of agar per plate, but the use of average petri dishes, a few with bottoms very slightly thicker in the center was justified by the spacing of the three discs equidistant from the center. Had this slight unevenness affected the area of inhibition, the diameter would not have been the same in two places. That is, the area would have been oblong or at least not a circle and all areas were circular throughout the period of work. Uniform seeding of the Staphylococcus as the test organism was maintained by inoculating 50 ml. of 1% peptone broth in a small Erlenmeyer flask, incubating for eighteen to twenty-four hours and planting into the melted agar by means of a wire loop.

Diffusion of the antibiotic substances through the solidified agar, especially when the petri dishes were inverted during incubation, could only be kept as constant as possible by uniform volume of agar and adhering rigidly to the formula in its preparation. A second factor which might have affected the diffusion of the antibiotic substances through the agar was the inversion of the petri dishes during incubation. This was believed necessary to prevent the condensate on the inner surface of the larger half from dropping on the agar surface. There was no special attention given to the use of perfectly dry petri dishes because at the time the melted agar was poured, there was always a condensation developed whether or not one had existed prior to that operation. Since the testing discs were placed on the solidified agar not later than one-half hour after pouring, it was

believed impractical to arrange a drying procedure and possibly induce contamination during the process.

Because there was no way of diluting the antibiotic substances in this method of testing, the only alternative was to reduce the amount per disc by the use of smaller discs. Larger discs were found to be unsatisfactory because of overlapping areas of inhibition during the peak of antibiotic activity.

CONCLUSIONS

1. *Pseudomonas culture 34* and *Pseudomonas culture 51* exhibit antibiotic activity although the average area of inhibition over a ten weeks period was not so great as that of *Pseudomonas aeruginosa.*

2. There is a distinct parallel between the first pigment production and the first antibiotic activity in certain cultures, but this is not found to be true in others.

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A STUDY OF ABUNDANCE OF STEMS PER ACRE IN RELATION TO AGE OF STAND[1]

By CHARLES D. GRIFFIN

Of the great numbers of seeds and spores which are produced annually by the plant kingdom, only a relatively small number germinate, and fewer still are able to withstand the tremendous competition for light, soil moisture, nutrients, etc. Only a small number of seedlings are able to ecese. Weaver and Clements (8) state that competition in effecting the supply of water and light is most decisive during the development of the seedling and at the time of reproduction, particularly among perennials and woody plants. Accordingly it plays a large part in determining the number of occupants and of invaders in an area in each stage of a sere and thus in helping to control the course of development.

From studies and data collected on forest succession, several facts have been determined. Potzger and Friesner (4) state that in a mature forest, the wide spacing of trees indicates the great mortality among young trees which germinated in the shade and under the stress of increased demand on the products of photosynthesis and water find the habitat lacking in adequate supply of light and moisture. Of course one sees an undercover growth of small trees, but these are tolerant secondary species and hide the real mortality among the seedlings of the dominant species controlling the crown cover. Burns (3) states that the establishment of a forest depends upon the numbers which survive rather than the numbers germinating. Turner (7) says that many trees succumb to competition, and the number of trees which come to maturity may be an exceedingly small percentage of the original number of young trees in a given area. Potzger and Friesner (4) found that the successional stages are characterized by the decreasing total number of species as the climax forest is approached. These facts and the statement by Turner (7) that there are relatively few quantitative data on the annual or period reduction in the number of trees in developing for-

[1] This is contribution 200 from the Botanical laboratories of Butler University.

ests are the basis for the undertaking of this study. Grateful recognition is made of the aid given in the field work by members of the Ecology class of 1947. Thanks are expressed to Dr. J. E. Potzger for suggestions, help in the field work, and critical reading of the manuscript.

PURPOSE

The purpose of this study is to gain a concept of the number of stems growing on an area in relation to the age of the stand, and to discover to some extent how the process of elimination of individuals proceeds.

METHODS

The number of stems 3 feet high and over in 9 different half-acre plots was tabulated, and the average of the stems in each plot was determined. These tabulations are compared with one another in relation to the age and the number of stems. These tabulations are also discussed and compared with the available data of other experiments relating to this subject.

The data collected for this study are from two general habitat types, i. e., upland and lowland river valleys. Results are presented in seven tables, three for tabulation of stems in one-eighth acre plots in upland woods, three for tabulation of one-eighth acre plots in floodplain woods, and one in summary form showing the various size-classes for easy comparison.

RESULTS

In the upland woods, soil moisture is probably the greatest limiting factor to tree succession, while on the floodplain, light would be the greatest limiting factor. This is evident in a comparison of stands of the two types of habitats. Where there is an abundance of soil moisture, the stands growing on the floodplain show a definitely greater number of mature trees per acre than the stands on the upland. The mature floodplain stands presented in tables V-A and III both have a total of 96 stems per acre over 10 inches DBH., while the two stands on uplands (tables I and VII-A) have respectively 72 and 56 stems per acre over 10 inches DBH. However, the number of small stems per acre in the upland woods is greater than in the stands of the floodplain woods. The mature upland stands (tables

I and VII-A) have 528 and 224 stems per acre with a DBH. of 1 to 5 inches, while the mature floodplain stands (tables V-A and III) had 48 and no stems of the 1 to 5-inch DBH. size-class. This must in part be due to the number of understory species represented in the two locations. The two upland stands were represented by 13 and 11 species, of which 7 and 4 were second layer and immature dominant tree species (Carpinus, *Cornus florida, Celtis occidentalis, Fraxinus lanceolata, Fraxinus quadrangulata, Prunus, Morus rubra, Ulmus americana, Ulmus fulva* and *Vitis*), while the two floodplain woods were represented by 7 and 9 species respectively, of which only one tree species (Cornus florida) was of the second layer type. In a study of mature upland woods, Auten (1) found an average of 19 species represented, and an average of 222 stems per acre of the 1 to 5-inch size-class, and 55 tree stems per acre over 5 inches DBH. As to the character of the understory, he found that some were dense and some were sparse. The two mature upland stands included in this study were represented by 80 and 88 stems per acre over 5 inches DBH.

The number of stems which are able to mature is relatively small in comparison with the number of seedlings which get a start in a given area. In this study it is found that there is an immense difference in the number of stems per acre between a young stand and an older or mature stand. Two young upland stands (tables II and VII-B) show a total of 1752 and 1856 stems respectively per acre of one inch DBH. and over, while the two mature upland forests (tables I and VII-A) show a total of 608 and 312 stems respectively per acre of one inch or over DBH. Two young stands on the floodplain (tables V-C and IV) have totals of 1760 and 4448 stems of one inch and over DBH. per acre, while the two mature floodplain stands (tables II Iand V-A) have 280 and 216 stems respectively.

These differences in numbers are large, but when only certain species are recorded, the significance of the mortality rate is more clearly shown. When all species are included in the count, the subdominant species tend to hide the truth as to the number of trees which actually survived the competition from the beginning of the sere. Table VII shows the differences in number of the individual species from one-eighth acre plots of mature (A) and young (B) upland stands. *Acer saccharum* shows a total of 1472 stems per acre in the young stand and 352 in the mature stand. *Carya ovata* shows

even a greater decrease, with 80 stems per acre in the young stand and only 8 in the mature stand. *Liriodendron tulipifera* is represented by 104 stems per acre in the young stand, and by 40 stems in the mature stand. Such species as Carpinus, *Cornus florida* and *Fraxinus lanceolata* show a marked increase in the number of stems per acre in the mature stands as compared with the young stand. Since these species are more or less tolerant, their greater number of stems in the mature stands are probably due to the fact that they do not have the close competition of the dominant species, as they do in the young stand. One species, *Fagus grandifolia,* is a dominant tree, but its representation in the young stand is only 8 stems per acre, while in the mature forest it has 40 stems per acre. It is possible in this case that the proper conditions for the germination of beech seeds had not been reached in the young successional stage.

Table V is the tabulation of species in three different one-eighth acre plots on the floodplain. The mature stand is designated by "A," the intermediate by "B," and the youngest by "C." *Acer negundo* has 584 stems per acre in the young stand, 264 in the intermediate, and 104 in the mature stand. *Acer saccharinum* presents an even greater decrease of stems with increase in age of the stand. It is represented by 656 stems per acre in the young stand, 80 in the intermediate, and only 32 in the mature forest. *Ulmus americana* is represented by 2832 stems per acre in the young stand, and by only 56 stems in the mature stand. *Ulmus thomasi* has 464 stems per acre in the young stand and only 72 stems in the mature forest. *Platanus occidentalis* is not represented in either the young or intermediate stands but has 32 stems per acre in the mature stand. This condition is similar to the absence of Fagus seedlings in the young stand and the greater number of stems found in the mature upland forest of this study.

Table VI presents the number of dead stems present in the three one-eighth acre plots on the Fort Harrison floodplain. In the young stand (C) there were 1208 dead stems per acre of under 1-inch size-class, and 3672 stems living. In the intermediate stand (B) were 152 dead stems one inch or over in diameter, and 328 living. In the mature stand were 24 dead stems and 216 living. In the mature forest, 8 dead stems per acre with DBH. of 5 inches or over were found, as compared with 168 stems of this size-class living.

DISCUSSION

The influence of age on characteristics of a forest stand, especially as to number of stems per acre, has had considerable attention by foresters and ecologists. The associated phenomena and the factors operating in the struggle centered about the elimination of individuals still warrant additional consideration based on quantitative data.

Morosow, as quoted by Braun-Blanquet (2) recorded approximately 419,464 ten-year-old beeches per acre in a Russian forest, 1,784 fifty-year-old stems per acre and 203 stems of beeches 120 years of age. In a stand of red maple and white pine, Burns (3) recorded 225 seedlings the first year, and after ten years, only 4 had survived. In a study of shortleaf pine, Turner's data (7) on the number of stems per acre from a 9-year-old to a 152-year-old stand is revealing as to the great decrease in the number of stems per unit area in relation to the increasing age of the stand. In the 9-year-old stand of shortleaf pine, he found 19,008 stems, while with only a difference of seven years, a 16-year-old stand had 8783, or less than half the number of the younger stand. A 35-year-old stand had 1638 stems per acre, a 40-year-old stand had 744, a 60-year-old stand had 415, a 70-year-old stand had 180, a 116-year-old stand had 120 stems, and the 152-year-old stand had only 80 stems per acre.

In their gross features, results of the present investigation echo those of the workers just referred to. The factor of competition initiates elimination of numbers of individuals until an equilibrium is established between the carrying capacity of a given habitat and the number of stems in such an area. Habitat demands involve both aerial and edaphic factors, as the present study plainly shows. In the two forest types which were investigated (upland and floodplain), the aerial phase of the habitat features are more or less identical, especially with respect to light, but the soil moisture of the edaphic group is strikingly different. In the floodplain forest, soil moisture plays a secondary role in the elimination process, as shown by the summary data presented in table VIII. A mature floodplain forest has from 25 to 40 per cent more stems per acre than a comparable stand of upland forest. Stevens (6) found that the less soil moisture available, the sooner the roots will occupy the available soil, and the sooner competition between individuals will begin. Mortality rate is a mirror which reflects the process of elimination in action, for dead

stems are impressive mute evidence of struggle for survival. Numbers of dead stems is inversely proportional to the age of the stand, and the absence of small size-classes in older stand mortality figures reflect cessation of abundant reproduction (table VIII). The struggle for survival is also recorded by the repression in diameter growth of trees constituting two age groups (tables V-B, V-C). The groups presented here are separated by a difference in age of about 5 years. They have, however, so striking a difference in diameter-size distribution as to suggest a much greater difference in the age between the two stands. The large numbers of dead stems in the group "C" stand suggests also a more rapid elimination of individuals after about ten years of growth than after that period. Of course, numbers of dead stems should decrease with continuance of elimination.

SUMMARY AND CONCLUSION

1. The paper presents data on abundance of stems per acre in similar stands of forest types, but ranging in age from ten years to a mature stand.

2. A mature floodplain forest has from 25 to 40 percent more stems per acre than a mature upland forest.

3. The thought is expressed that the cause for elimination in a floodplain forest is primarily the light factor, while in upland forests, it is a combination of light and soil moisture deficiency.

4. The number of small stems per acre in the mature upland woods is greater than in the stands of the mature floodplain woods.

5. When only certain species are recorded, the significance of the mortality rate is more clearly shown.

6. The number of dead stems in three one-eighth acre plots on the floodplain is presented.

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TABLE I

Upland mixed mesophytic climax forest. One-eighth acre plot.

Species	Size Classes in Inches								Total Stems
	Under 1	1	2	3-5	6-10	11-17	18-20	Over 21	
Acer saccharum	25	25	10	1	1		2		64
Carpinus caroliniana		1							
Carya ovata	1						1		2
Cornus florida			1	1					2
Celtis occidentalis		7	4	1					12
Fagus grandifolia			1			4		1	6
Fraxinus lanceolata		2	1	1					4
F. quadrangulata		1							1
Ostrya virginiana	1					1			2
Prunus		2							2
Ulmus americana		1	2	3					6
Virburnum prunifolium	2								2
Vitis			1						1

TABLE II

15-year growth of upland maple of secondary succession. One-eighth acre plot.

Species	Size Classes in Inches										Total Stems
	Under 1	1	2	3	4	5	6	7	8	9	
Acer saccharum		52	79	41	16	5	1	1			195
Fraxinus lanceolata	2	1									3
Gleditsia triacanthos							2				2
Ulmus americana					2		2	1			5
U. fulva				1	2	2	6	2	2	1	16

TABLE III

Mature floodplain forest. One-eighth acre plot.

Species	Size Class in Inches							Total Stems
	Under 1	1	2	3-5	6-10	11-17	18-20	
Acer negundo	2	1			1	4	1	9
Celtis occidentalis	5	2		1				8
Cornus florida	1							1
Fraxinus americana						1		1
F. quadrandulata	20				2			22
Juglans nigra	1							1
Ulmus americana			1	8	6	6		21
Sambucus canadensis	1							1
Vitis	3	1						4

TABLE IV

Floodplain young stand (15 years) of elm of secondary succession. One-eighth acre plot.

Species	Size Classes in Inches											Total Stems
	Under 1	1	2	3	4	5	6	7	8	9	10	
Acer negundo	1	6										7
A. saccharinum							1					1
Cornus florida	1	2										3
Celtis occidentalis	3	1										4
Prunus		1										1
Ulmus americana	2	3	2	1								8
U. fulva	1		1	2	1		2				3	10
U. thomasi	12	185	105	62	24	30	8	4		1	1	532
Vitis	3	6	4	1								14

TABLE V

Floodplain mature stand (A), intermediate (B), and young stand (C). One-eighth acre plots.

Species (Living)		Size Classes in Inches								
		Under 1	1	2	3-5	6-10	11-17	18-20	Over 21	Total Stems
Acer negundo	(A)	10			3					13
	(B)	2			21	9	1			33
	(C)	37	16	9	9	2				73
Acer saccharinum	(A)					1		1	2	4
	(B)			1	4	3	2			10
	(C)	17	29	28	8					82
Aesculus glabra	(A)									
	(B)									
	(C)	1								1
Celtis occidentalis	(A)									
	(B)									
	(C)	1		1	1					3
Cornus asperifolia	(A)									
	(B)									
	(C)	49	27							76
Crataegus	(A)									
	(B)									
	(C)	53	32	1						86
Fraxinus americana	(A)									
	(B)									
	(C)	2	1							3
Platanus occidentalis	(A)						2		2	4
	(B)									
	(C)									
Prunus	(A)									
	(B)									
	(C)		1							1
Rosa	(A)									
	(B)									
	(C)	11								11
Sambucus	(A)	6								6
	(B)	3								3
	(C)									

TABLE V—(Continued)

Species (Living)		Size Classes in Inches								
		Under 1	1	2	3-5	6-10	11-17	18-20	Over 21	Total Stems
Smilax hispida	(A)	2								2
	(B)									
	(C)									
Ulmus americana	(A)				3	1	3			7
	(B)									
	(C)	265	73	15	1					354
Ulmus fulva	(A)									
	(B)									
	(C)		1	1	1					3
Ulmus thomasi	(A)					5	4			9
	(B)									
	(C)		35	22	1					58
Vitis	(A)									
	(B)									
	(C)	32	5							37

TABLE VI

Floodplain dead stems in mature stand (A), intermediate stand (B), and young stand (C). One-eighth acre plots.

Species (Dead)		Size Classes in Inches								
		Under 1	1	2	3-5	6-10	11-17	18-20	Over 20	Total Stems
Acer negundo	(A)									
	(B)		4	6	5					15
	(C)	18	6	1						25
Acer saccharinum	(A)									
	(B)		2							2
	(C)	13	3							16
Cornus asperifolia	(A)									
	(B)									
	(C)	4								4
Crataegus	(A)									
	(B)									
	(C)	2								2
Ulmus americana	(A)									
	(B)		1							1
	(C)	94	7							101
Ulmus thomasi	(A)			1	1	1				3
	(B)		1							1
	(C)	20	4							24

TABLE VII

Upland study of Cox's woods: mature stand (A); young stand (B). One-eighth acre plots.

Taken from report by Potzger, Friesner and Keller. (5)

Species		Size Classes in Inches								Total Stems
		Under 1	1	2	3-5	6-10	11-17	18-20	Over 20	
Acer saccharum	(A)	20	3	5	15	1				44
	(B)	7	118	39	20					184
Asimina	(A)									
	(B)	9								9
Carpinus	(A)	13	1							14
	(B)		1							1
Carya ovata	(A)					1				1
	(B)		1		6	3				10
Celastrus	(A)									
	(B)	3								3
Cornus florida	(A)	3								3
	(B)		1							1
Crataegus	(A)									
	(B)				1					1
Fagus grandifolia	(A)		1	2					2	5
	(B)		1							1
Fraxinus lanceolata	(A)	57	1							58
	(B)	4								4
Juglans nigra	(A)					1				1
	(B)									
Liriodendron tulipifera	(A)							1	4	5
	(B)	1	4	3	2	3				13
Morus rubra	(A)	1								1
	(B)									
Prunus serotina	(A)									
	(B)		1							1
Quercus velutina	(A)									
	(B)		1							1
Rhus	(A)									
	(B)		11	15						26

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TABLE VII—(Continued)

Species		Size Classes in Inches: Under 1	1	2	3-5	6-10	11-17	18-20	Over 20	Total Stems
Ulmus americana	(A)					1				1
	(B)									
U. fulva	(A)	3								3
	(B)									

TABLE VIII

Summary of significant data. Compiled from tables I to VII.

	Age of Stand	Number of Species	Under 1 Inch	1-inch and Over	1-5 Inches	Over 5 Inches	Over 10 Inches
LIVING							
Upland, Table I	Mature	13	232	608	528	80	72
Upland, Table VII-A	Mature	11	776	312	224	88	56
Floodplain, Table V-A	Mature	7	144	216	48	168	96
Floodplain, Table III	Mature	9	264	280		168	96
Floodplain, Table V-B	25 years	3	40	328		120	24
Floodplain, Table IV	15 years	9	192	4448		160	
Upland, Table II	15 years	5	16	1752		144	
Upland, Table VII-B	Young	14	192	1856		48	
Floodplain, Table V-C	9-12 yrs.	13	3672	1760		16	
		*	*	* *	*		
DEAD							
Floodplain, Table VI-A	Mature	8		24	16	8	
Floodplain, Table VI-B	25 years	4		152	152		
Floodplain, Table VI-C	9-12 yrs.	6	1208	168	169		

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